RESPONSES OF THE WEED *DIGITARIA ABYSSINICA* (A.Rich.) Stapf TO SELECTIVE GRASS HERBICIDES IN UGANDAN COTTON.

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ABSTRACT

Successful weed control is essential for economical cotton production in Uganda. Weeds compete with cotton for moisture, nutrients and light. The greatest competition usually occurs early in the growing season. Therefore, post emergence herbicides such as fluazifop-butyl and sethoxydim were used in this study to control the grass weed species which grow faster than cotton during its initial growth stage. Field trials were conducted on the sandy loam/loam soils at Namulonge Research Institute and Bukalasa Technology Verification Centre in Uganda during the 1995/96 and 1997/98 cotton seasons to investigate the control of the tropical couch grass (*Digitaria abyssinica* (A.Rich.) Stapf) using the post emergence herbicides. This weed is a rhizomatous grass and difficult to control. The efficacy of the reduced dose rates of the grass selective post emergence herbicides sethoxydim and fluazifop-butyl was investigated for the control of *D. abyssinica* and other grass weed species in cotton so as to determine the appropriate dose rate(s). The herbicides were supplemented with two hand weedings. Hand weeding (5 times) during the growing season was included in the treatments.

The results obtained from the field trials showed the potential of reduced dose rates in the control of grasses. The application of fluazifop-butyl (138, 162 and 188 g a.i.ha^{-1}) and sethoxydim (405, 502 and 579 g a.i.ha^{-1}) gave a significant density reduction of *D. abyssinica* and other annual grasses at 35 days after herbicide application. No significant differences were observed amongst the dose rates of both herbicides in the percentage weed control of *D. abyssinica* and other grass weed species in the two seasons. The percentage weed control ranged between 79-96%. The assessment showed that fresh and dry weights of *D. abyssinica* shoots/foliage were reduced by 70-80% irrespective of the dose rates for both herbicides at the two sites during the two seasons.

Reduced dose rates below half of the full dose rates were investigated in the greenhouse in UK. A markedly reduction of *D. abyssinica* shoots and rhizomes was noted following the application of fluazifop-butyl (38, 66, 94, 188 g a.i.ha^{-1}) and sethoxydim (116, 203, 290, 579 g a.i.ha^{-1}) compared to the control. An average percentage reduction of 43.2-62% for fresh and dry shoots, and 65.9-78% for fresh and dry rhizomes was observed. Although analysis of variance indicated that there were no significant differences amongst dose rates, low percentage reduction was noted from the lowest dose rate of fluazifop-butyl (38 g a.i.ha^{-1}).

As part of the plant stress assessment, results indicated that sethoxydim and fluazifop-butyl inhibited chlorophyll accumulation in the treated leaves of *D. abyssinica*. A reduction of 40-70% of chlorophyll content was noted. It was noted that sethoxydim had higher inhibitory effect on chlorophyll content than fluazifop-butyl. Results obtained from the measurements of fluorescence parameters showed significant reduction of Fv/Fm in the leaves of *D. abyssinica* due to sethoxydim and fluazifop-butyl, suggesting significant alteration of the normal fluorescence yield. Interference with the normal fluorescence of *D. abyssinica* was associated with the inhibition of the flow of electron transport which resulted to plant stress. Further investigation on plant stress was studied by comparing the activity levels of the intracellular proteases of *D. abyssinica* and cotton plants after the application of sethoxydim. The activity level of alanyl aminopeptidase was not affected by the herbicide both in *D. abyssinica* and cotton. It was however noted that there was significant decrease in the activity levels of argninyl aminopeptidase (arginyl-ap) (36.1%) and tripeptidyl aminopeptidase (Tap) (51.8%) in the treated plants of *D. abyssinica* with time after herbicide application. While in cotton, significant increase was observed in the activity level of and tripeptidyl aminopeptidase with time after herbicide application. Therefore it can be assumed that the activity levels of these enzymes in the two plant species may have an influence on their responses to the herbicide.

Data on crop performance indicated that seedcotton yields realised from herbicides dose rates combined with two hand weedings were high as or higher than the yields obtained from the hand weeding (5 times). Seedcotton yields were noted high (1793-2993 kg/ha) in 1995/96 compared to 1997/98 (665.1-1184.2 kg/ha). The present study has indicated that integration of reduced dose rates with two hand weeding supplements can reduce the number of weedings and improve cotton production.

This thesis is dedicated to my late loving father, and my mother, my daughter Kate and the entire family.

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DECLARATION

This work reported in this thesis is based upon the author's own research studies under supervision of Dr. Richard M Wilkins in the Department of Agricultural and Environmental Science at the University of Newcastle upon Tyne. No part of this material offered has been submitted previously for a degree or other qualification in this, or any other University.

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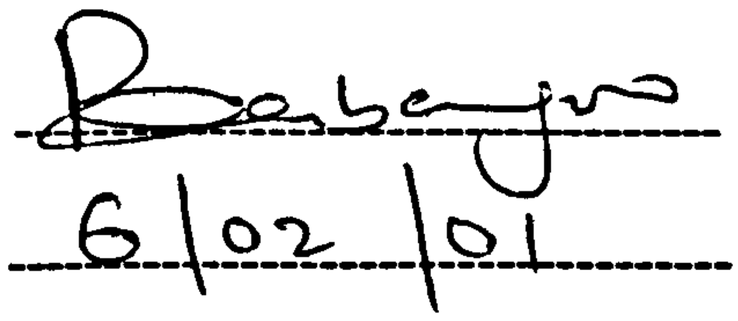

ABBREVIATIONS

a.i	active ingredient
cm	centimetre
EC	emulsifiable concentrates
Fifi	dirty seedcotton
Fo	minimum fluorescence
Fv	variable fluorescence
Fm	maximum fluorescence
Fv/Fm	ratio of variable fluorescence and maximum fluorescence
g	gram
dap	days after planting
h	hour
ha^{-1}	per hectare
kPa	kilo pressure
mm	millimetre
m	metres
me	milligram equivalent
$m.s^{-1}$	metres per second
m^2	metre square
mg/g	milligram per gram
ml	millilitre
nm	nanometre
Safi	clean seedcotton

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Origin of cotton

Cotton (*Gossypium hirsutum* L.) is the world's leading fibre crop and belongs to the family *Malvaceae*, genus *Gossypium* (Hutchinson *et.al* 1947; Fryxell 1979). The crop is reported to have originated from Central Mexico and spread to the Southern United States (Smith, 1968). To date 33 million hectares of the crop are grown in 75 countries world-wide (Lavabre, 1991). According to FAO (1994) statistics, cotton production is highest in Southern USA and least in Europe and Australia. Cotton production world-wide has varied greatly in response to fluctuating world market prices. On the whole production has been in decline, partly because of an increase in utilisation of synthetic fibres which is estimated to have increased by 60% in 1994/95 compared to 1987/89 (FAO, 1994). World-wide, the USA is the leading exporter of cotton (ICAC, 1993).

1.2 Cotton production in Uganda

Cotton was introduced into Uganda in 1903 as a cash crop. It was brought to the country when the Uganda Cotton Company imported half a ton of the cotton-seed, thereafter a series of introductions followed. In Uganda, two commercial cotton varieties are grown, BPA (Bukalasa Pedigree Albar) and SATU (Serere Albar Type) are grown in the two different agroecological zones. BPA is grown in the wet zones in the central and Western regions while SATU is grown in the drier areas of the eastern and northern regions. For a long period cotton remained the leading foreign exchange earner for the country until 1950 when it was overtaken by coffee. From 1920 to 1940 cotton earnings sustained at least 60% of the entire population of Uganda (Omoding, 1994). Between 1966 and 1970s

cotton exports averaged 63.6 million metric tonnes and Uganda ranked third after Egypt and Sudan in cotton production in Africa (Omoding, 1994). Cotton production in Uganda steadily decreased after 1971 and it was lowest in 1981 when only 2000 metric tons were produced. The decline was attributed to poor marketing, political instability and neglect of cotton research.

1.3. Production constraints

1.3.1. Insect pests

In Uganda cotton production is constrained by insect pests, pathogens and weeds (Hillocks, 1995). More than 1000 species of insect pests are reported to attack the cotton crop world-wide (Hargreaves, 1948). Of these, the most important are early season pests which affect establishment of the crop and may lead to 100% loss (Matthews *et al* 1972). In Uganda the cotton aphid (*Aphis gosypii*), the american boll worm (*Heliothis armigera*), cotton stainers (*Dysderus* spp) and cotton lygus (*Lygus hesperus*) are reported to be of major economic significance (Bowden and Thomas, 1970).

Attempts to reduce the effect of pests in cotton production have included breeding for varietal resistance, selective chemical and biological control, diverse cultural measures and recently integrated pest management (IPM) (Matthews, 1989). In Uganda, chemical control has been the main method of pest control. However, recently, IPM has been emphasised by the Smallholder Cotton Rehabilitation Project (SCRP) (El-Heneidy, 1994), to try and reduce on the number of chemical sprayings, combined with cultural methods such as intercropping cotton and beans.

1.3.2. Common diseases

Diseases are of equal economic importance to insect pests in cotton production, (Snowden, 1926; Hansford, 1929; Hansford, 1932; Hansford, 1935; Bird, 1959; El-Nur, 1970; Hallion, 1983; Blasingame, 1990). Major cotton diseases include bacterial blight (*Xanthomonas malvacearum*) and wilt diseases such as *Fusarium oxysporum*, *Verticillium dahlia*, *Ramularia areola* and *Nematospora gospii* (ICAC, 1993). Bacterial blight (*Xanthomonas malvacearum*) is the most important disease in cotton production in Uganda. It is suspected to have originated from India (Knight, 1948), although it was first reported in the USA (Atkinson, 1891). At almost the same time bacterial blight was reported in Uganda (Hansford, 1932).

1.3.3. Weed species

Weeds represent one of the most severe constraints to yields, usually causing greater losses than other pests. However, their significance is easily overlooked unlike that of insect pests and diseases. Although weeds are less emphasised in most cotton growing systems in developing countries, they are reported to result in yield reduction if left unchecked (Keeley and Thullen, 1989; Vencill *et al.*, 1992). Singh *et al.*, (1996) reported 30% crop losses due to weeds alone, compared to a 16% loss due to pathogens and 12% due to insect pests. However, yield reduction depends a lot on the weed type, density and agro-ecological conditions.

Cotton plants are sensitive to weed competition because they grow relatively slowly in the early growth stages. Cotton does not attain full ground cover until eight or more weeks

after germination. Thus it is important to control weeds during the early stages of cotton growth, which is quite laborious with hand-hoe weeding only. In various countries a lot of work has been carried out to determine the most critical period for weed control in cotton. In the Sudan, for instance Crowther (1943) reported the seventh and eighth week after planting to be the most critical growth period while Thomas (1969) considered the first six weeks to be the most critical period. Thomas and Schwerzel (1968) found that two to four weeks after crop emergence was the critical period in Zimbabwe.

Major weed species in cotton fields in Uganda include *Digitaria abyssinica* (A.Rich.) Stapf, *Rottboellia cochinchinensis* (Lour), *Imperata cylindrica* (L.) Raeuschel, *Cyperus* spp., *Panicum maximum* Jacq, *Cynodon* spp., *Eleusine indica* (L.) Gaertn, *Digitaria velutina* (Forssk), *Commelina* spp., *Amaranthus* spp., *Ageratum conyzoides* (L.), P.Beauv., *Bidens pilosa* L., *Galinsoga parviflora* Cav. and *Tagetes minuta* L. (Webb *et al.*, 1993).

To date there is limited understanding of the effect of weed species in cotton production in Uganda. *Digitaria abyssinica* is a troublesome weed in most cotton fields in Uganda (Prentice, 1957; Harker, 1957; Webb *et al.*, 1993). *D. abyssinica* is a difficult weed to eradicate by cultivation alone. Forked hoes have been used to remove the rhizomes, but total eradication of the small viable fragments in the soil has been virtually impossible (Terry, 1974). Terry (1974) added that this method is both time consuming and costly. Prentice (1957) emphasised chemical control of *D. abyssinica* for complete eradication. He also noted that scanty *D. abyssinica* was controlled with relatively high concentrations

of dalapon. He however, suggested that the economics of complete eradication of heavy infestation of *D. abyssinica* should be studied. However, Hocombe (1960) and Baguma (1995) observed that when *D. abyssinica* is chemically controlled, there are many other annual weeds which sprout. They are usually easy to control, except in a few cases where *D. abyssinica* is replaced by *Cyperus* spp. which is also troublesome weeds. Because of its importance in most cropping systems in Uganda, this weed was studied in the present study. Its control by hand-hoeing, temporarily checks the weed due to its rhizomes. To adequately control this weed, systemic chemicals/herbicides were suggested by Mwakha, (1974); Mwakha, (1979); Wanjala, (1995). Therefore, fluazifop-butyl and sethoxydim were used to control *D. abyssinica* in this study.

1.4 *Digitaria abyssinica*

1.4.1 Distribution

D. abyssinica is widely distributed in the moisture regions of East Africa from sea level to 3000 m. It is a common component of the natural grasslands at higher altitudes. *D. abyssinica*. It is the most troublesome weed which occurs in a range of crops such as coffee, tea, sisal, pyrethrum, cotton and many other annual and perennial crops in Kenya, Tanzania and Uganda and it is also present in Ethiopia, Malawi, Somalia, Sudan and Zambia (Terry and Michieka, 1987). In South Africa it has been introduced into the Transvaal and Natal. It has been planted in the Cape of GoodHope Peninsula where it forms a thick turf on the mountain slopes (Huxley and Turk, 1966).

1.4.2 Biology of *D. abyssinica*

Digitaria abyssinica, sometimes called *Digitaria scalarum* (Schweinf.) Chiov.), is commonly known as couch grass. It is a perennial grass weed that belongs to the family Gramineae or Poaceae. *D. abyssinica* produces long and slender rhizomes, forming a dense mat beneath the soil surface. Its rhizomes can penetrate to a depth greater than one metre. The rhizomes are made of short nodes and short internodes, with the roots rising from the nodes. Any small fragment of a rhizome with a node is capable of producing a new plant once left in the ground (Harker, 1957). Harker, (1957) suggested that multiplication of this weed by seed is not considered as a method of propagation. On the other hand, Holm *et al.*, (1977) reported the contribution of seed to the spread of *D. abyssinica*. Although *D. abyssinica* is of significant importance in most crops in East Africa, its biology has not been studied in detail (Prentice, 1957). Attempts to study the biology of this weed were done by Otieno (1967). He reported that due to air spaces in the root cortex, *D. abyssinic*a can adapt to water logged soils.

1.4.3 *Digitaria abyssinica* as a weed

With a heavy infestation of *D. abyssinica*, both growth and yield of crop plants are tremendously reduced (Ivens, 1967 and Mbevi, 1997). It is regarded as the most troublesome weed of arable land in some parts of East Africa (Otieno, 1967).

Like any other tropical rhizomatous grass, *D. abyssinica* grows well under high light intensity. Higher infestation of this weed is normally observed in unshaded fields rather than shaded ones. Once established *Digitaria* rhizomes penetrate throughout the soil, including the middle of the root system of the crop plants. Hence it becomes difficult to remove them without the use of herbicides. When removing rhizomes by cultivation,

there is a danger of damaging the roots of the crop plants. However, Hocombe (1960) and Baguma (1995) observed that when *D. abyssinica* is chemically controlled, there are many other annual weeds which sprout. They are usually easy to control, except in a few cases where *D. abyssinica* is replaced by *Cyperus* spp. which are also troublesome weeds.

D. abyssinica has the potential to cause major crop losses. A survey conducted by Webb *et al.*, (1993) indicated much about the high yield losses of more than 50% which was associated with *D. abyssinica* in various crops. According to Baker and Terry (1991), yield reductions of the order 7, 12, and 24% at weed population of 3, 20, and 177 plants m^{-1} respectively were reported for maize. Reduction of yields in various crops due to *D. abyssinica* has been noted. In Uganda Prentice, (1957) noted that *D. abyssinica* reduced the yield of cotton by 50% or more if the weed is left unchecked. Similar observation with a bean crop showed greater yield loss due to *D. abyssinica.* High yield reductions in sisal were reported by Holm *et al.*, 1977). Wallis (1959) gave evidence that the effects of this weed on coffee could partly be due to production of toxic exudates. He further noted that the weed might also be affecting other crop species through an allelopathic action.

1.5 Weed control methods in cotton

Various methods of weed control exist, but the choice depends on correct weed species identification, the site, the crop infested, techniques used to grow the crop and level of farming. These methods include physical, cultural biological and chemical methods, legislative quarantine, and the integrated weed management approach (Crafts, 1975, Klingman *et al.*,1982; Roberts, 1982; Anderson, 1983). A wide range of weed species are

commonly managed or controlled through cultural, chemical, and more recently integrated pest management (IPM) (or integrated weed management -IWM). This approach is of advantage in the fact that for many pests, no single method can effectively solve all problems related to a particular pest group. Thus a range of options for weed control are being integrated in various ways of producing crops. On the other hand, however, when one control method is used alone, success of such method usually requires high input levels. For example in the case of cultural control, the high inputs may be reflected in high costs of control, while with chemical control the high input is not only in form of high costs but also at the risk of pesticide residue in the environment.

1.5.1. Cultural weed control

This is a weed management practice which uses all aspects of good crop husbandry to reduce weed-crop competition (Akobundu, 1987a). Smallholder farmers in most developing countries commonly practise this method. The aspects of crop husbandry include hand-hoeing or weeding/pulling, cultivation or inter-row cultivation with animal-drawn weeders, mowing, mulching, flooding and crop rotation.

1.5.1.1. Cultivation

Cultivation can be done by both animals and tractors to enable farmers to efficiently use power driven implements (Stout *et al.*, 1973). Such a practise to be used for weeding, can increase farm produce, improve farmer's income through increased economic returns, reduce farm labour drudgery and enables the farmer to weed large acreage per unit time compared to when he would do manual weeding. On the other hand, cultivation is done to improve the physical and nutritive condition of the soil and to control weeds (Zimdahl,

1980, van Rijn, 1982). Cultivation loosens the soil, incorporates the above ground vegetation into the soil and increases the soil porosity (van Rijn, 1982). Increased soil porosity can be used as temporary water storage during rainfall and may improve infiltration. Cultivation controls weeds by exposing the weed seeds on the soil surface where they can easily germinate once the conditions are favourable (Roberts and Nelson, 1981). The seeds are left to germinate because the weed seedlings are easily controlled by hand-hoe weeding or any other means of control. As suggested by Terry, (1984) several cultivations could probably control *D. abyssinica,* but it needs to be studied to determine the frequency and interval of cultivation. To obtain maximum weed control it is important to start weeding at the onset of the rains. However, Butters and Clegg, (1963) observed nitrate leaching at the beginning of the rains in the absence of plant cover. On the other hand, cultivation is associated with disadvantages which include drying of the soil surface which leads to high risk of wind or water erosion, especially where soil conservation measures are not practised (Lal, 1976; Kemper and Derpsich, 1979) as cited by van Rijn, (1982). In addition, cultivation can only efficiently control annual weed species, while rhizomatous perennial weed species such as *D. abyssinica* can not be easily be eradicated, especially where tillage is used as a weed control method involving animal drawn implements.

1.5.1.2. Use of cover crops

Ground cover crops have been used as a weed control measure in crops such as coffee and other tree crops (Douglas and Hart, 1976; Ruthenberg, 1976). According to van Rijn (1982), various legume cover crops such as beans suppressed *D. abyssinica* in coffee plantations. In Uganda the use of cover crops as a weed control measure is not practised

on a large scale, although recently it has been practised in agroforestry. Legume cover crops mainly beans are used in the intercropping systems of cotton and other crops for food production but not as a measure for weed control. In the Ivory coast, it was reported that ground cover crops used in cotton significantly reduced weed density and hence led to the reduction in number of weedings (Deat, *et al.*, 1977). Elsewhere, long lasting weed control with legume green covers in cassava was reported by Leihner (1980). He further observed weed control with mulches, although he noted that it was a short term weed control measure. A combination of live mulches (legume cover crops) and organic mulch (grass and banana/plantain leaves) was studied by Oladokun (1978). He noted that the organic mulch was more effective than the live mulches. Live mulches for weed control in tree crops was also reported by van Rijn (1982). Mulches for weed control in cotton production in Uganda are not yet commonly used by the cotton farmers.

1.5.1.3. Use of plant population/ spacing (crop density)

Manipulation of plant population/spacing (crop density) can also reduce weed growth. As reported by Shetty and Krantz (1980), plant density of Sorghum and Pearl millet (180,000 ha^{-1}) suppressed weeds in India. But in Zambia the same crops were reported to have reduced weed growth at a plant density of 100,000 ha^{-1} (Boringher, 1987). The use of plant population/spacing of various crops has been reported to have adquately suppressed the weeds. These include soya beans(*Glycine max*) (Felton,1976); faba bean (*Vicia faba* L.) (Nassib *et al.*, 1982); peas (Ahmed, 1983); mung beans (Santi *et al.*, 1991); cassava (Manihot esculenta) (Baguma, 1997). The use of plant population/spacing for weed control in cotton production has been investigated.

Walton (1962) recommended high plant density in cotton for suppression of weeds in Uganda. Rogers *et al.* (1976) also indicated that narrow spacing in cotton reduced the number of weedings compared to wide spacing. In Uganda however, high cotton plant populations and narrow spacings might not be applicable due to the fact that areas where soil fertility is high, this practice might affect the crop performance in terms of number of bolls per plant and seecotton yields.

1.5.1.4. Hand weeding

Hand weeding is the oldest method of weed control which consists of hand pulling, hand slashing, hand hoeing and weed mowing. The time spent weeding varies amongst crops, depending on the cropping pattern, spacing or spatial arrangement of crops and the age of the weeds. Weeding cotton and other crops in Uganda is traditionally done by hand weeding, and approximately 40-80% of the farmers' time is consumed by this method (Miller, 1982; Koch *et al.*, 1982; Ocitti-P'obwoya, 1985). In addition, this practise is associated with labour drudgery in subsistence farming and as a result timely weeding not usually done. In another study in India, Gill (1982) reported that 200-400 man-hours in a season were required to weed an established cotton crop. In most farming systems high weeding labour requirement can limit the acreage (Armitage and Brook, 1976, Koch *et al.*, 1982). Reichelderfer (1984) added that once labour required for weeding is reduced, more acreage might be acquired and hence more income generated. Parker (1977), Compton (1982) and Gill (1982) suggested improvement on the design of hand tools to increase their efficiency and reduce the effort required by the labourer. This

might be a relief to the women who do most of the weeding in most crops in developing countries. According to URT (1993) women are largely, if not solely responsible for crop production in Tanzania. Field operations (land preparation, weeding and harvesting of annual crops) are their responsibility in addition to their other household chores. Women and children contribute as much as 70-90% of the labour force which is allocated to weed control practices. In general terms, hand weeding has limited agricultural productivity because there is a limit to the amount of acreage that can be weeded manually despite the free labour.

1.5.2. Biological weed control

Biological weed control is a method used to suppress weeds using one or more organisms by manipulating the weed, organism or the environment (Anonymous, 1985). Successful biological weed control was obtained through the use of vertebrate (Schmidl, 1977; Zon, 1984), and invertebrate animals (macrobial control) (Batra, 1981; Delfosse and Cullen, 1981; Julien, 1982), microorganisms such as plant pathogens (microbial control) (Harris, 1981; Templeton and Smith, 1982; Templeton, 1983) and live mulch (Akobundu, 1984a). According to MüllerSchärer and Frantzen, 1996, this approach is suitable where only one single weed species is dorminant in a crop, and where immediate or complete weed control is not required. Furthermore in situations where weed problems in agro-ecosystems are not caused by single weed species, biological control has to be considered as an integrated component in pest management strategy and not as a single control method. According to MüllerSchärer *et al.*, (1999), although microbial herbicides rapidly kill the weed target, combinations of biological control with other weed management practises might be needed for acceptable levels of overall weed control.

Watson and Wymore, (1989), termed such integration as either a vertical integration of various control tactics against a single weed species or a horizontal integration across different weed species in one crop. In Uganda, studies conducted on the biological control of the aquatic weed Waterhyacinth (*Eichhornia crassipes)* have showed good results on the control of this weed with weevil species *Neochetina eichhorniae* and *Neochetina bruchi* (Ogwang and Molo, 1997). Although this control method was successful for the control of the waterhyacinth, it hasn't been commonly applied to control other weed species in short term crops such as cotton nor in long term crops such as coffee. This might be due to the fact that there is always a wide range of weed species in the field.

1.5.3. Chemical weed control

1.5.3.1 Herbicide classification

According to Akobundu, (1987b), herbicides consist of inorganic, organic and biological herbicides. On the other hand, herbicides are classified depending on the time of application (pre or post emergence), point of application (foliage or soil), plant species (selective or non-selective), physiological action (photosynthetic inhibitors, pigment inhibitors, respiration inhibitors etc), movement in plants (contact, translocated or systemic herbicides) and chemical or composition of the active ingredients (Probst *et al.*, 1975; Bartels and Watson, 1978; Ashton and Crafts, 1981; Fedke, 1982; Roberts, 1982; Harper and Appleby, 1984). However, most herbicides currently used in agriculture world-wide are organic herbicides. These herbicides became popular because they control most problematic weeds. Some of them control only annual weeds whereas others control both annual and perennial weeds.

Organic herbicides consist of a number of groups amongst which are aryloxyphenoxy pronionic acid and cyclohexanedione to which fluazifop-butyl and sethoxydim respectively belong (Akobundu, 1987b)

1.5.3.2. Herbicide activity against *D. abyssinica* and other weed species

Once in the plant, herbicides affect plants in many ways depending on how they are applied, plant species and the environment or conditions under which the two are brought into contact. Most herbicides however, are known to interfere with a metabolic pathway when applied to plants, in the process the herbicides may affect cell division (mitosis) (Carwright, 1976, Deal and Hess, 1980, Deal *et al.*, 1980). Most herbicides such as fluazifop-butyl and sethoxydim have been noted amongst those herbicides that affect cell division (Delvin *et al.*, 1985). Although a wide range of herbicides do kill plants, a few such as 2,4-D stimulate plant growth when applied at sublethal concentrations. Control of *D. abyssinica* in various crops has been carried out using herbicides. Successful results to control *D. abyssinica* with Na-TCA in sisal were reported by Richardson (1967) while Mitchell (1969) recommended the use of dalapon in coffee. In his greenhouse studies Parker (1970) noted good control of *D. abyssinica* with asulam. In a subsequent (personal communication) investigation, Parker (1970) found that glyphosate was active on *D. abysinica*. In other studies the activity of glyphosate on *D. abyssinica* has been reported by Butters and Clegg (1963); Baird *et al.* (1971); Terry (1974); Arnold *et al.* (1976); Onsando *et al.* (1989); Njoroge and Kimemia (1993) and Baguma (1995). They supported the research findings by Baird *et al.*, (1971) who had studied the activity of glyphosate against perennial grasses.

In Uganda dalapon was successfully used to control *D. abyssinica* but at high rates of application (Arnold *et al.,* 1976). This approach was not environmentally friendly and it was costly for smallholder farmers. Although a lot of herbicides have been used to control *D. abyssinica*, there is yet little documented on promising herbicides such as sethoxydim and fluazifop-butyl for the control of *D. abyssinica* in cotton in Uganda. Therefore there was a need for a study on the possibility of controlling this weed with sethoxydim and fluazifop-butyl in cotton, specifically, the role of dose rates lower than the recommended rates needed attention since there is concern about agrochemical residues in the environment and food supply system(s). This would at the same time initiate investigation on the costs of herbicides which has been a major concern for most cotton smallholder farmers. The forking out of *Digitaria* rhizomes has sometimes been practised as an another attempt to reduce the number of weedings. In this case the number of weedings can be reduced if reduced herbicide dose rates are combined with one or two weedings instead of weeding 4 or 5 times throughout the season. However a few cotton farmers do apply glyphosate during land preparation so as to reduce weeding labour. Research elsewhere has showed that the application of herbicides to the soil or foliage for weed control in a pure stand of cotton is widely adopted (Vencill *et al.*, 1992; Frans *et al.*, 1988; Carter and Keeley, 1987; Stevens *et al.,* 1986; Haitas *et al.*, 1995; Perry, 1994; Tillman *et al.*, 1986). This is not yet a common practice in cotton production in Uganda. However, post emergence herbicides would be preferred in Uganda to pre-emergence herbicides, since smallholder farmers can not attain a fine seedbed during land preparation. Farmers also like to see the problem before they do anything about it.

Application of a number of post emergence herbicides on various problematic weed species has been widely studied elsewhere. Keeley *et al.*, (1984) reported good control of johnson grass (*Sorghum halepense*) with glyphosate. Glyphosate usually gives good weed control of most annual and perennial weed species but because of its non –selectivity action, it can not be applied in established crops especially annuals. In this case other post emergence herbicides such as fluazifop-butyl and sethoxydim can safely be applied in cotton for grass control (Akobundu, 1987c). The use of sethoxydim and fluazifop-butyl for weed control in cotton has been reported in various countries (Bryson, 1985; Harden and Stewart, 1985; Makhan'kova *et a*l., 1987; Panwar *et al.*, 1988; Jordan *et al.*, 1993). Weed control in cotton, like any other crop, results in increased cotton yields and good lint quality due to reduced weed-crop competition. El-Deeb *et al.* (1984) reported high cotton yields and increased cotton fibre length when weeds were controlled with a tank mixture of fluometuron and fluazifop-butyl in cotton. Studies conducted elsewhere indicated that control of weeds in cotton with fluazifop-butyl and sethoxydim contributed to promising yields (Keeley *et al.*, 1987; Byrd and York, 1987; Panwar *et al.*, 1988; Keeley and Thullen, 1989; Keeley and Thullen, 1991; Haitas *et al.*, 1995).

1.5.3.3. Mode of action of fluazifop-butyl and sethoxydim

Fluazifop-butyl and sethoxydim are post emergence and grass selective herbicides. These herbicides have been widely applied on a range of grass weed species, this therefore led to their selection for the control of *D. abyssinica* and other annual grass weed species in cotton. In addition, they are highly systemic and very active against annual and perennial grasses in broad-leaved crops such as cotton, soybean, sunflower, peanuts potatoes and vegetables (Chandrasena and Sagar, 1987; Asare-Boamah and Fletcher, 1983).

On the other hand, sethoxydim and fluazifop-butyl have been reported to provide consistent weed control under favourable conditions and crop tolerance to these herbicides is high (Harker and O'Sullivan, 1991).

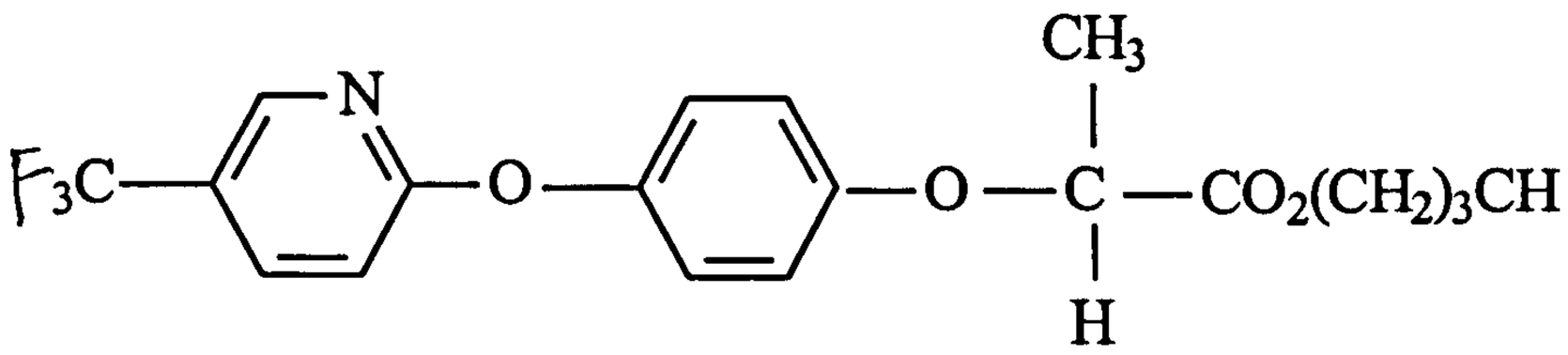


Figure 1.1.Structure of fluazifop-butyl

Fluazifop-butyl and sethoxydim are translocated in the plant through the xylem and phloem to metabolic sinks (Plowman *et al.*, 1980; Campbell and Penner, 1981). Their mobility within susceptible plants has been studied (Chandrasena and Sagar, 1984, Kells *et al.*, 1984). Sethoxydim and fluazifop-butyl have also been associated with the reduction of chlorophyll, damage on the chloroplast ultrastruture and inhibition of cell division in the meristems of the susceptible plants (Swisher and Corbin, 1982; Fletcher and Kirkwood, 1982; Asare-Boamah and Fletcher, 1983; Gealy and Slife, 1983; Chandrasena and Sagar 1984; Magallanes *et.al.*, 1986; Chandrasena and Sagar, 1987; Wakeham and Kirkwood, 1991; Hallgren and Fischer. 1992; Maligeppagol *et al.*, 1994). Fluazifop is applied as a ester, and its activity against the plant requires the accumulation of the active compound at the growing sites. Its molecular weight is 383.4, and molecular formula is

$C_{19}H_{20}F_3NO_4$. The herbicide is reasonably stable in acidic and neutral conditions, but rapidly hydrolyses in alkaline media (Negre *et al.*, 1988). It is soluble in water at 1mg/L pH6.5, and it can easily mix with acetone, cyclohexanone, hexane, methanol, dichloromethane and xylene. Its stability is up to 3 years under storage conditions of 25^{0}C, but at high temperatures (37^{0}C) it can be stable for only 6 months. Fluazifop-butyl is usually hydrolysed into a fluazifop acid in the plant (Balinova and Lalova, 1992). Fluazifop is the active form of the herbicide that is translocated and accumulates in the meristems, rhizomes and stolons of grasses such as *Elymus repens*, *Setaria viridis* L. Beauv (Hendley *et. al.*, 1985; Carr, *et al.*, 1986; Chandrasena and Sagar, 1987; Balinova and Lalova, 1992). The mode of action of this herbicide is not yet well understood. However, inhibition of fatty acid synthesis following its application has been studied, mainly in green plant tissues. Chloroplasts isolated from susceptible barley and maize were susceptible to fluazifop acid compared to chloroplasts isolated from resistant pea species (Walker *et al.*, 1988; Hoppe and Zacher, 1985). Inhibition of fatty acid synthesis in the chloroplasts was associated with the inhibition of the target enzyme acetyl-CoA carboxylase (EC 6.4.1.2) which is located in the chloroplasts. It is an essential enzyme for plant growth and development because of its key role in the fatty acid and lipid synthesis (Ohlrogge and Browse, 1995). Acetyl CoA Carboxylase in plants has been studied in different tissues (Heinstein and Stumpf, 1969; Brock and Kannangara, 1976; Kannangara and Stumpf, 1972; Nielsen *et al.*, 1979). Soluble preparations of the enzyme have been obtained from wheat germ (*Triticum* sp.) (Hatch and Stumpf, 1961; Heinstein and Stumpf, 1969), barley embryos (*Hordeum* sp.) (Brock and Kannangara, 1976), and avocado (*Persea americana*) (Mohan and Kekwick, 1980).

According to Post-Beitten Miller *et al*, (1991) and Harwood, (1991), any changes in the activity of acetyl CoA Carboxylase can be associated with light regulation of fatty acid synthesis. Acetyl CoA carboxylase inhibition following the application of fluazifop-butyl has extensively been studied (Herbert *et. al.*, 1996 Hidayat and Preston, 1997; Herbert *et. al.*, 1997). It is also known that aryl-propanoic acids to which fluazifop-butyl belongs, are antagonists of auxin activities in the plant. This is due to the availability of a free carboxyl group in the active herbicide molecule which enhances this interference which seems not to have any relationship with the inhibition of acetyl-CoA carboxylase (Olson and Nalewaja, 1981; Shimabukuro *et al.*, 1986). In animals such as rats and dogs, fluazifop-butyl rapidly hydrolyses and is excreted as fluazifop acid in the urine and faeces. Similar observation was noted for human beings, the acid was excreted following a single dose of 0.07mg kg^{-1} and an average of 88% was excreted within 6 days (Ramsey *et al.*, 1992). They further noted that the application of fluazifop-butyl on the skin was poorly absorbed (< 8% of the applied dose) which was rapidly excreted in urine. Degradation of fluazifop-butyl in soils was studied by Arnold *et a*l., (1982), who found that the herbicide slowly degraded in flooded soil. Its degradation in sandy loam and calcareous clay loam was reported by Bewick, (1986). According to Negre *et al.*, 1988, 21 days after application, 40% of the herbicide was found in the air-dried soil but in moisture soils the content was less than 5% after 7 days. They further noted a marked decrease of carbon dioxide evolution in the air-dried soil which was associated with degradative processes due to microbial activity.

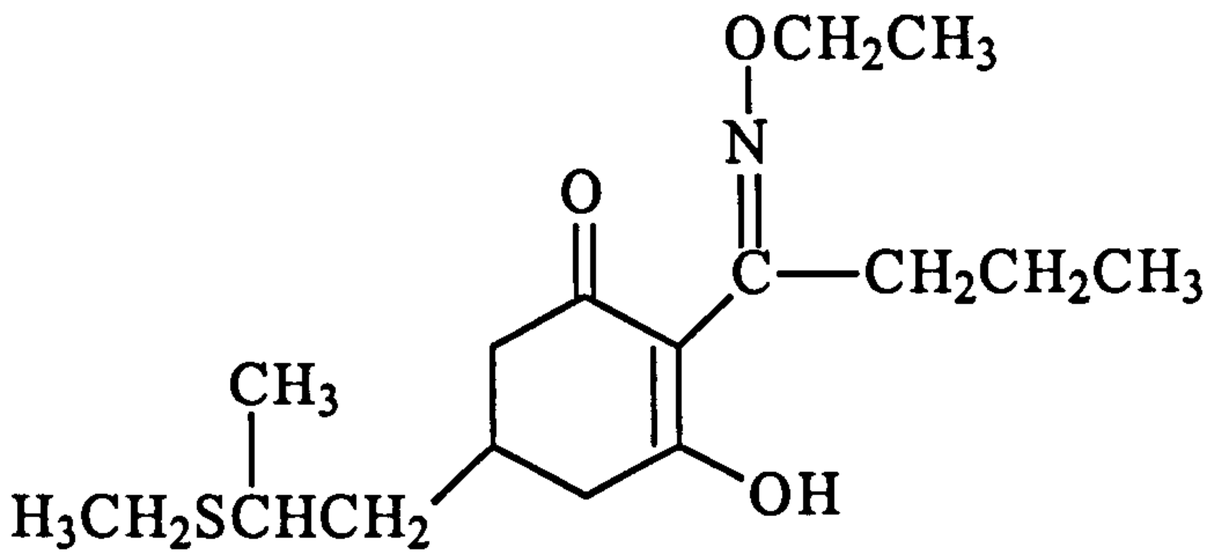


Figure 1.2. Structure of sethoxydim

The molecular weight of sethoxydim is 327.5 and its molecular formula is $C_{17}H_{29}NO_3S$. Sethoxydim dissolves in water at pH 4 in 25mg/L but its solubility drastically changes with the change in pH, for example at pH 7 it dissolves 4700mg/L (20°C). Sethoxydim is also soluble in most organic solvents such as acetone, benzene, ethyl acetate, hexane and methanol of 1 kg/kg at 25°C. Sethoxydim can be kept under normal conditions for 2 years at approximately 25°C. In the plant sethoxydim is transformed into a number of metabolites (Swisher and Corbin, 1982; Campbell *et. al.*, 1985; Shoaf and Carlson, 1986; Shoaf and Carlson, 1992). It is reported that this herbicide is degraded in susceptible grass species such as *Agropyron repens* L., *Echinochloa crus-galli* L., as well as tolerant species like *Phaseolus vulgaris* L. Seafarer (Campbell and Penner, 1985; Campbell and Penner, 1987). The pH in plant tissues ranges between 4-8 (Hosaka and Takagi, 1987), as a result sethoxydim degradation occurs *in vivo*, forming products that exhibit herbicidal activity of the chemical.

Other studies have showed that sethoxydim inhibits *de novo* fatty acid biosynthesis in susceptible plants such as maize (Ishihara *et al.*, 1986; Burgstahler and Lichtenthaler, 1984; Burgstahler *et al.*, 1986; Hatzios, 1982). It was also noted that fatty acid synthesis in tolerant soybean plants was inhibited by sethoxydim implying that the herbicide's selectivity might be due to its differential metabolism in plant species (Hatzios, 1982). On the other hand, it was reported that inhibition of fatty acid synthesis might be another way of giving detailed results on membrane disruption (Hoppe, 1980), chloroplast damage and loss of chlorophyll (Brezeanu *et al.*, 1976), CO_2 fixation (Köcher *et al.*, 1982), reduced ATP content and mitochondrial dysfucntion (Gronwald, 1986). Research findings however, give evidence that sethoxydim inhibits acetyl CoA carboxylase *in vitro* (Burton *et al.*, 1987; Secor and Cséke, 1988; Rendina and Felts, 1988; Herbert *et. al.*, 1997), which is known as the site of action of this herbicide (Gronwald, 1991; Secor *et al.*, 1989). In addition, the rate at which sethoxydim exerts its phytotoxicity might depend a lot on its stability in the environment (Hatzios, 1982). When sethoxydim was administered to rats, rapid elimination of 79% and 25% of the applied herbicide was noted in urine and faeces respectively within 48 hours. In soils, sethoxydim does not persist. In a study by Shoaf and Carlson, (1992), a recovery of less than 10% of the applied herbicide was obtained from the dry soil.

1.5.3.4. Measurements of plant stress due to herbicides

Herbicide action is usually known as the physiological and biochemical interaction of a herbicide with a plant. According to Devine *et al.*, (1993), this interaction is not only one but there are multiple interactions at various levels within the plant which interfere with the plant health resulting in plant senescence. Therefore photosynthetic parameters such as fluorescence can be measured, although photosynthesis might be a secondary site of action for some herbicides (Willard *et al* 1990).

1.5.3.4.1. Fluorescence

The measurements of fluorescence parameters have been extensively studied to effectively assess herbicide injury and accurately quantify photosynthetic inhibitor herbicides (Schreiber *et al* 1975, Richard *et al.*, 1983, Walker, 1985, Chandrasena and Sagar, 1987). These parameters can give fast and non destructive methods to evaluate herbicide activity (Schreiber *et al.*, 1977; Baker and Bradbury, 1982; Cadahia *et al.*, 1982; Richard *et al.*, 1983; Voss *et al.*, 1984). Light energy absorbed by chlorophyll molecules in the leaf is usually used in the photosynthetic process (photochemistry), can also be given away as heat, or re-emitted as light (chlorophyll fluorescence) (Maxwell and Johnson, 2000). This is illustrated in Figure 1.3.

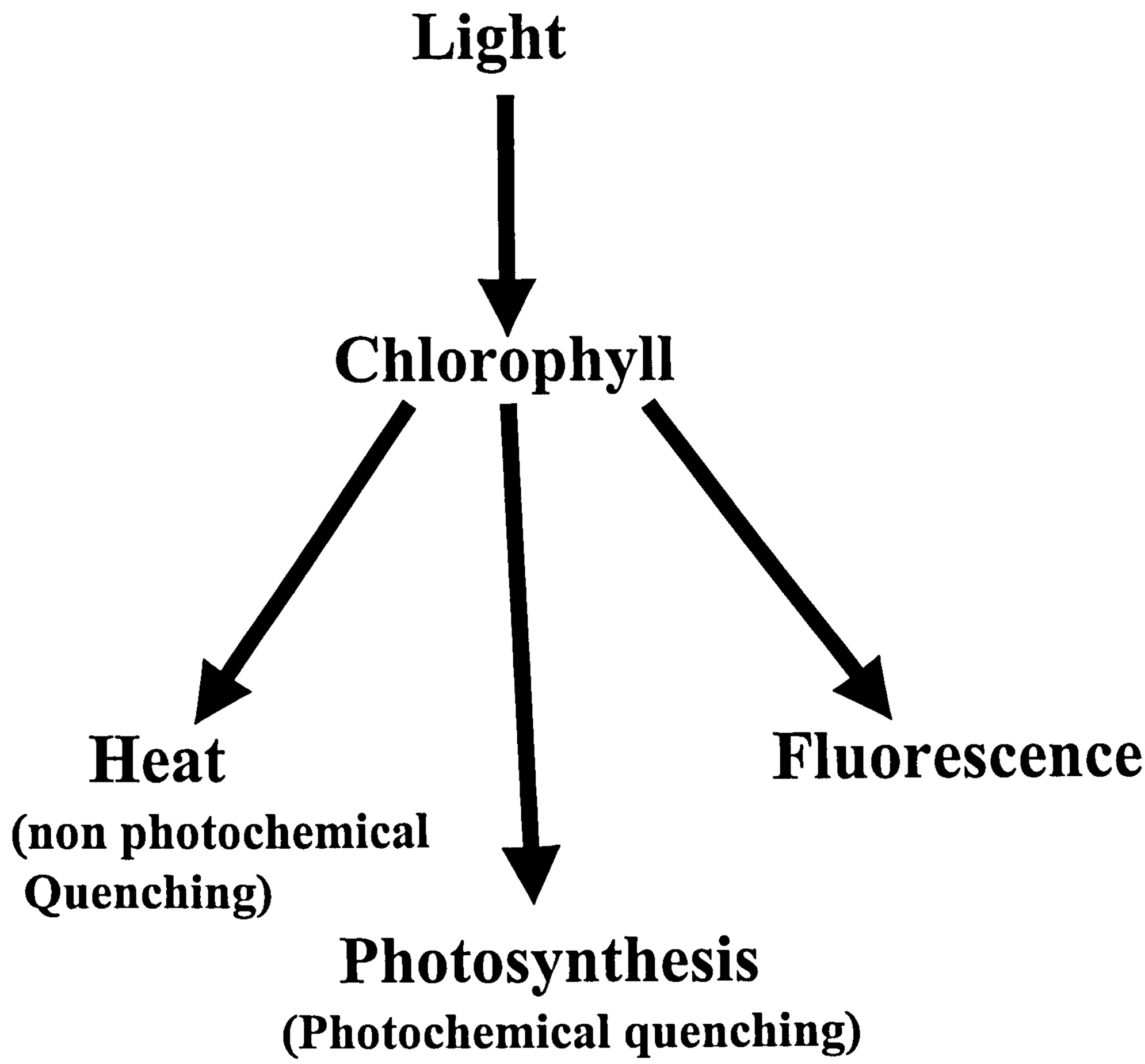


Figure 1.3. Light utilisation in the plant

Fluorescence parameters include Fo, Fv, Fm and Fv/Fm ratio. They are normally measured after dark-adaptation of the leaf. A leaf is irradiated with a constant flash of light after dark-adaptation, it exhibits a typical fluorescence known as the Kautsky effect (Schreiber and Bilger, 1987). Fluorescence initially rises from a minimal value (Fo) to a certain level or peak. It then gradually decreases almost to its original Fo through intermediates. This decrease in fluorescence is termed quenching (Krause and Weis, 1991). Fluorescence yield is minimal (Fo) when all reaction centres are oxidised or open and are available for photochemistry. Fm is known as the maximum total fluorescence obtained when the reaction centres are closed, while Fv is the maximum variable fluorescence, which is described as the change

in fluorescence between maximal and minimal fluorescence. This change reflects the reduction of the primary electron acceptor (Q_A) (Krause and Weis, 1984). The Fv/Fm ratio indicates the efficiency of excitation energy capture by the open reaction centres of PSII. Under optimal conditions the Fv/Fm ratios of various plant species were found to be around 0.83 by Demming and Björkman, (1987) and Johnson *et al.*, (1993). They further explained that if a plant is exposed to stress the values of Fv/Fm ratio can be lower than normal. Fluorescence can be used to assess herbicide injury to the plant before the visual symptoms occur. Herbicide penetration into the leaf tissues is an important component in herbicide activity. This level has been studied using fluorescence parameters (Habash *et al.*, 1985). Similarly assessment of weed resistance to herbicides has been done using fluorescence (van Oorschot and Straathof, 1988). Studies by Chandrasena and Sagar (1987) showed that measuring Fv/Fm can indicate the activity of post emergence herbicides. This was supported by Hubbard and Whitewell (1991) who observed reduction of Fv/Fm ratio after the application of fenoxaprop-ethyl and sethoxydim on the susceptible plants. Similarly fluazifop-butyl has been reported to have caused significant change Fv/Fm ratio of *Elymus repens* (L.) Gould leaves (Chandrasena and Sagar 1987). This indicates that this measurement can also be used to measure the injury of fluazifop-butyl and sethoxydim on *D. abyssinica.* According to Willard *et al.*, (1990) the inhibition of fluorescence quenching may depend a lot on structural integrity of the thylakoid membrane.

1.5.3.4.2. Proteases and protein

On the hand plant stress can be measured through investigating the activity level of plant proteases (Callis, 1995). Proteases are widely distributed in animal, insect and plant tissues but are best characterised in animals. The characteristics of many individual enzymes have been shown to be similar or identical in different tissues within and between various animal species (Mantle, 1992; Faiz *et al.*, 1994). The universal distribution of proteases in various tissues may probably reflect a fundamental role of these enzymes in the generalised process of intracellular protein degradation, a process which is essential for the normal functioning of all cells. Intracellular protein degradation has been recognised to involve several separate degradative pathways associated with specific subcellular organelles or protease types, including the lysosomal pathway (cathepsins), the Ca^{2+} dependant cytoplasmic pathway (calpains) and recently the abiquitin-proteosomal pathway (multicatalytic proteinase). Proteolytic activity in plants was reported as early as 1799 by Vauquelin, 1799. Proteases were isolated from fruits and latex of various plants, but not until of recent that proteases have been studied in cellular processes in plant metabolism. A lot of work was reviewed concerning proteases in plants with emphasis on the properties of enzymes such as papain (Arnon, 1970; Glazer and Smith, 1970), chymopapain (Glazer and Smith, 1970; Kunimitsu and Yasunobu, 1970), ficin (Glazer and Smith, 1970; Leiner and Friedenson, 1969) and bromelin (Murachi, 1971). In addition to these, a lot more other proteases of various plants have been extensively studied in germinating seeds and their activities have been associated with the seedling growth and development (Ryan, 1973).

According to Hartley, 1960, proteases are classified into four groups which are based on their site catalytic mechanisms. These include serine proteases, cysteine proteases, metallo proteases and aspartic proteases. Proteases can further be classified depending on their substrate specificities such as endopeptidase, carboxypeptidase or aminopeptidase. The endopeptidase and carboxypeptidase play a key role of breaking down the reserve proteins in germinating seeds. In general terms proteases can also be classified on basis of the pH optimum of activity (acid, neutral or alkaline). Some work done on cotton indicated an increase in proteolytic activity beginning with or at the onset of cotton seed germination (Ihle and Dure, 1969). Ihle and Dure, (1969); Ihle and Dure, (1972) noted that in cotton seed, carboxypeptidase was produced *de novo* 24 hours after germination and the increase continued up to the fourth day before it disappeared. It is therefore suggested that the endopeptidases found during the first stages of germination are responsible for the initiation of breaking down the stored proteins to polypeptides for further degradation and transport them to the growing embryo as peptides or amino acids. Similarly the proteolytic activity at germination of other seed crops has been extensively studied. These include barley (Bhatty, 1969; Jacobsen and Varnes, 1967, peas (Beevers, 1968), lettuce (Shain and Mayer, 1968) and sorghum (Garg and Virupaksha, 1970). According to Jacobsen and Varner, 1967, in barley *de novo* synthesis of endopeptidase activity rapidly increased during germination. They pointed out that in the germinating barley many other proteolytic enzymes were observed and their activities changed during development. However, recently proteases in cereals have received a lot of attention (Rastogi and Oaks, 1986; San Segundo *et al.*, 1990; Wrobel and Jones, 1992; Mitsuhashi and Oaks, 1994; Domminguez and Cejudo, 1995), although the proteolysis of grain development is not yet well understood.

It was suggested that there is a possibility, at a certain stage of development, proteases may be synthesised. For example mature grains of barley studied by Sarkkinen *et al.*, 1992; Wrobel and Jones, 1992), rice (Doil *et al.*, 1980), maize (Mitsuhasu and Oaks, 1994) and wheat (Belozersky *et al.*, 1989), were all found to contain proteases. Intracellular proteases are ubiquitous enzymes distributed in all living organisms, and they are responsible for processing intracellular proteins which are essential for normal functioning of the cells. On the other hand, protease activity in plants has been investigated to evaluate their role in plants that are resistant or susceptible to herbicides or insect attack. In studies conducted by Wilkins *et al.*, (1999), it was noted that the levels of neutral and acidic proteases were significantly higher in resistant weed species compared to the susceptible ones (personal communication). Starrant and Lazarovits, 1996 noted high levels of free acids in herbicide-induced pathogen resistance in a tomato plant but it was not clear whether their increase resulted from *de novo* synthesis or increased proteolysis. The herbicide/pathogen resistance, enzymes/inhibitors may also influence the development of plant resistance to insect attack. According to Bowles, (1990); Ryan, (1992); Schaller and Ryan, (1996), plant proteases or proteases inhibitors can locally be induced by signals generated in response to herbivory infestation. For example a wounded tomato plant was observed inducing protective proteins/enzymes such as leucine aminopeptidase, aspartic proteases and cysteine protease inhibitors (Mcgurl *et al.*, 1992; Hildmann *et al.*, 1992; Hansen and Hannapel, 1992; Walling *et al.*, 1995; Constabel *et al.*, 1995). In respect to herbicides *per se*, protease activity is usually disturbed depending on the plant species, period after treatment and probably type of herbicide. Kumar and Prakasii, 1994 have clearly reported the activity of proteases in rice and barnyard grass after the application of thiobencarb and butachlor.

Metolachlor decreased the activity of proteases in tomato (*Lycopersicon esculentum* L.) seedlings (Gabr *et al.*, 1988). Other herbicides such as 2,4,5-T, dalapon, atrazine and bromoxynil were also observed affecting the protein synthesis in vivo and enzymatic activity of proteases of various plants (Tonecki, 1975a; Tonecki, 1975b; Hagemann, 1984). Tonecki, (1975a) noted that stimulation or retardation of protease activity due to dalapon depended a lot on herbicide concentration, plant growth stage and time of application. The information reviewed clearly shows that there is little known about protease activity in cotton and *D. abyssinica* plants, thus it was investigated in the present study.

1.5.3 5. Reduced dose rates of herbicides

The efficacy of herbicides such as fluazifop-butyl, sethoxydim and others has been evaluated by their respective agrochemical companies for the control of weeds under various conditions, thus recommended dose rates are given. However, in some cases these recommended rates are so high that they sometimes cause plant injury of some plant species. In a study conducted by Dexter, (1994), he found out that when desmedipham was applied at high dose rate of 1.12 kg ha^{-1} injured sugerbeet compared to when it was applied at low dose rate of 0.56 kg ha^{-1}. He further reported that the application of reduced dose rates of desmedipham and phenmedipham controlled the broadleaf weeds and resulted in less sugarbeet (*Beta vulgaris*) injury. Therefore reduction of herbicide consumption would be another way of reducing plant injury. This can be done through developing weed management strategies that prevent herbicides from getting to non targets. In this case the use of low rate concept could be one of the weed management strategies. Reduced or sublethal dose rates are herbicide dose rates that are reduced to a

certain percentage of the recommended rate at the time of registration. Some of the advantages of reducing dose rates are to reduce crop injury and reduce costs per acre or hectre. Therefore, rather than to continue using high rates which were recommended at registration, it would be better to encourage lowering the rates. This is also in consideration that the farmers are not the same world-wide, for example the objectives of the farmer in a developing country with regard to available farmland or number of people to be fed may differ from those of a farmer from a developed country. It is therefore important to make farmers occupy the central part of any recommendations made so that they don't find it impossible to produce food/cash crops in equal quantity and quality. In addition, Zoschke, (1994) concluded that any weed management technique considered suitable has to be adapted to the specific regional situation which in turn is influenced by society, environment and economics. The activity of reduced dose rates of various herbicides has been widely studied. Dexter, (1994) noted successful results from reduced dose rates of desmedipham for weed control in sugar beet. Trifluralin and pendimethalin applied at reduced dose rates were reported to have reduced weed dry weight (Panwar *et al.*, 1993). Vargas and Wright, (1993) reported the significance of reduced dose rates of metham for the control of weeds in cotton. Reduced dose rates of herbicides such as glyphosate (2 kg a.e.ha^{-1}) have also been reported to have successfully controlled *D. abyssinica* (Baguma, 1995). However, low levels of other herbicides such as sethoxydim and fluazifop-butyl have not yet extensively been used for the control of *D. abyssinica*. But studies elsewhere have showed good weed control results of various grass weed species with low levels of fluazifop-butyl and sethoxydim (Harden *et al.*, 1984; Whitewell and Brown, 1984; Panwar *et al.*, 1988; Bridges and Chandler, 1987; Manju-Nath *et al.*, 1990).

In other studies, it has been reported that the activity of fluazifop-butyl reduced dose rate of 53g ha^{-1} was improved by adding a wetting agent (Dowling and Nicol, 1993). Another study elsewhere indicated activity enhancement of low levels of fluazifop-butyl (0.28, 0.56 kg ha^{-1}) by adding crop oil (2.35 L ha^{-1}) and surfactant (0.25% v/v) (Graber and Hensley, 1987). While the addition of crop oil to low levels of sethoxydim (0.28, 0.56, 0.84 kg a.i.ha^{-1}) was reported by Mccarty (1983). These studies have confirmed the important role played by additives to improve the use of herbicide low levels. In the present study however, no additives were used to enhance the activities of fluazifop-butyl and sethoxydim.

1.5.3.6. Environmental problems of herbicides

Currently the use of chemicals in agriculture is being discussed at length. This is due to the growing concern about herbicide residues in foodstuffs, soil, ground water and atmosphere (Ellis, 1992; Watson, 1992). Herbicides have been viewed as environmental contaminants and a lot of public and private organisations are scrutinising the use of pesticides (Abernathy, 1992). According to Zoschke (1994), the only way to minimise herbicide consumption is to introduce low rate chemistries, low rate concept, innovative formulations, application timing and cropping systems approaches. As a result this study was initiated to control *D. abyssinica* with reduced herbicide levels.

1.5.4. Integrated weed management approach

Environmental issues have initiated the development of integrated weed management systems to minimise the impact of crop production on the environment. This is an approach which includes all aspects of cropping system, such as cultural and chemical, cultural and biological, cultural and preventive, biological and chemical or, combining three or more methods. A crop like cotton takes 6-7 months to mature, consequently one weed control method might not adequately control the weeds for such long period. The use of chemical in weed control supplemented with hand weeding in cotton was reported by Hamdoun (1979). He noted high cotton yields due to the effect of the combinations and observed reduction of man-hours required for hand weeding. A similar observation was noted by Muruganadam and Ali (1986) on the integration of oxyfluorfen with one hand weeding. Pendimethalin combined with hand weeding and a tank-mixture of diuron and pendimethalin combined with hand-hoe weeding were evaluated in cotton by Panwar *et al* (1991), Ramesh *et al.*, (1993) and Panwar *et al.*, (1993). Owing to high labour demand in cotton production, integrated weed management would be the only solution.

Other studies on integrated weed management have been conducted in most crops. These include groundnuts, (Mahalle, 1992), beans (Boller *et al.*, (1992); corn (*Zea.mays*), (Wilson, 1993); potatoes, (*Ipomoe batatas)* (Ridder *et al.*, 1993), and soya bean, (Chandel *et al.*, 1995) and others. Integrated weed management for the control of *D. abyssinica* has been studied. Baguma (1995) noted that a combination of glyphosate with slashing and burning effectively controlled *D. abyssinica*. In another study on *D. abyssinica*, Ngugi (1980) reported good control of this weed with a combination of good land preparation and chemical weed control.

But the integration of fluazifop-butyl and sethoxydim at different dose rates for the control of *D. abyssinica* has not yet been extensively documented.

1.5.5. Aims and objectives

Hand weeding is a laborious practice in farming systems of developing and developed countries (Chancellor, 1994). In fairly heavy soils annual weeds can be buried deep and they are eventually used as compost. In stony soils some weed species might not be efficiently buried thus they are forked out. Perennial weeds such as rhizomatous *D. abyssinica* can only be suppressed by systemic herbicides such as fluazifop-butyl and sethoxydim (Mwakha, 1974; Mwakha, 1979; Wanjala, 1995). But in order to protect the environment and achieve crop production with low inputs, reduced rates of fluazifop-butyl and sethoxydim were studied. The general objective of this study therefore was to determine the appropriate dosage rate(s) of fluazifop-butyl and sethoxydim for the control of *D. abyssinica* in cotton.

Specific objectives were;

1) to compare the efficacy of reduced dose rates with the recommended rates.

2) to investigate whether the weed stress after treatment is due to the loss of chlorophyll content or inhibition of fluorescence quenching.

3) to compare the intracellular proteases activities in cotton and *D. abyssinica* plants, and determine whether they play any role in the susceptibility or resistance of the two plant species.

CHAPTER TWO

The control of *D. abyssinica* with reduced dose rates of fluazifop-butyl and sethoxydim in cotton under field conditions.

2.1 Introduction

Cotton competes poorly with weeds such as *D. abyssinica* which is difficult to control by cultivation only. The critical period of cotton-weed competition is usually during the first 4-5 weeks after emergence (Street *et a*l., 1985). This clearly indicates the necessity of the early season weed control in cotton. However, it is also necessary to suppress the late season emerging weeds although they are not as competitive as the early season weeds but they may interfere with insecticide applications and probably may cause harvesting problems and the weed seeds contaminating the seedcotton. *D. abyssinica* can be associated with high yield losses. During heavy infestation of this weed, all cotton plant parameters are affected including biomass, plant height, stem diameter/ fruiting branches, and number of bolls per plant. The traditional hand-hoe weeding of this weed is labour demanding, sometimes smallholder farmers weed late due to labour pressure which results to weed-crop competition. It would therefore be necessary to identify weed control method(s) which are economic and time saving. This can be done through a combination of various methods of weed control (Swanton and Weise, 1991). According to Gebhardt *et al.* (1985), they noted that ineffective weed management is a limiting factor in crop production systems. The present study investigated the control of *D. abyssinica* with reduced dose rates of fluazifop-butyl and sethoxydim. The two hand weeding supplements included in this study were done to control the broad leaf weed

species which are tolerant to the grass selective herbicides. As part of the integration of weed management in cotton, the grass weed component of the weed population and the response of these weeds to doses below the full rates was investigated. The objectives of the field study were;

1) to investigate on the efficacy of the reduced dose rates of fluazifop-butyl and sethoxydim for the control of *D. abyssinica* in the field in Uganda.

2) to determine cotton yield components in response to the weed control combinations.

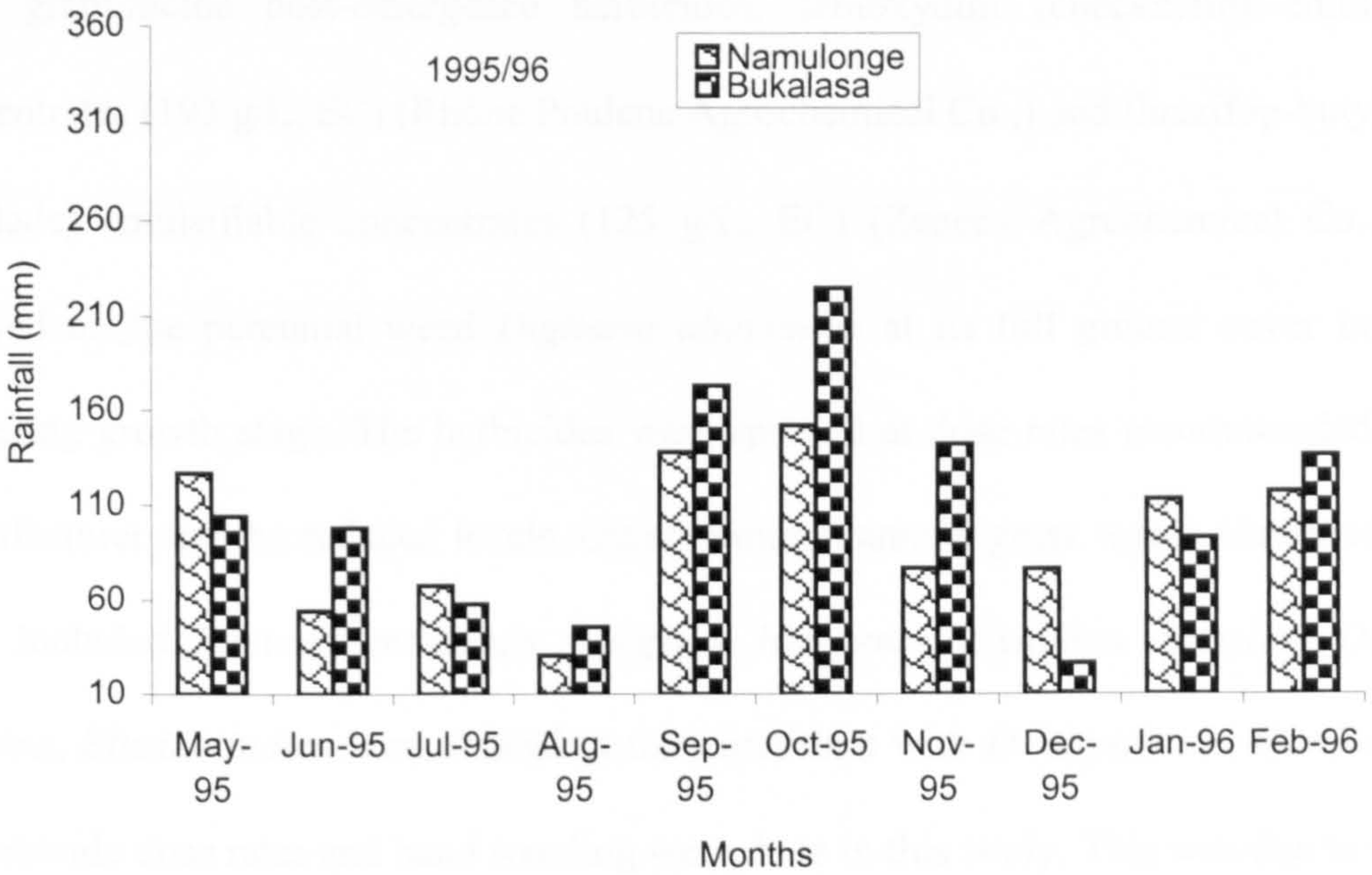
2.2 Materials and Methods

2.2.1. Experimental sites

Field experiments were conducted in Uganda at Namulonge Agricultural and Animal Production Research Institute (32^0 35' E) and Bukalasa Technology Verification Centre (32^0 31'E). Both sites are situated within the Lake Victoria basin which has a bimodal type of rainfall and an average of 1000-1270 mm per annum, minimum temperature is 21.3°C and relative humidity is 80 percent. The rainfall distribution during the experimentation season is illustrated in Figure 2.1. The soils at Namulonge are black-brownish sandy clay loam with low levels of phosphorus (PO_4) estimated at 4ppm and an average pH of 6. Bukalasa soils are dark-brown sandy loam with an average pH of 5.9 (Jameson, 1970).

2.2.2. Treatments and experimental design

The experiments were conducted during the 1995/96 and 1997/98 cotton seasons. The treatments are described in Table 2.1 and they applied in a randomised complete block design (RCBD).



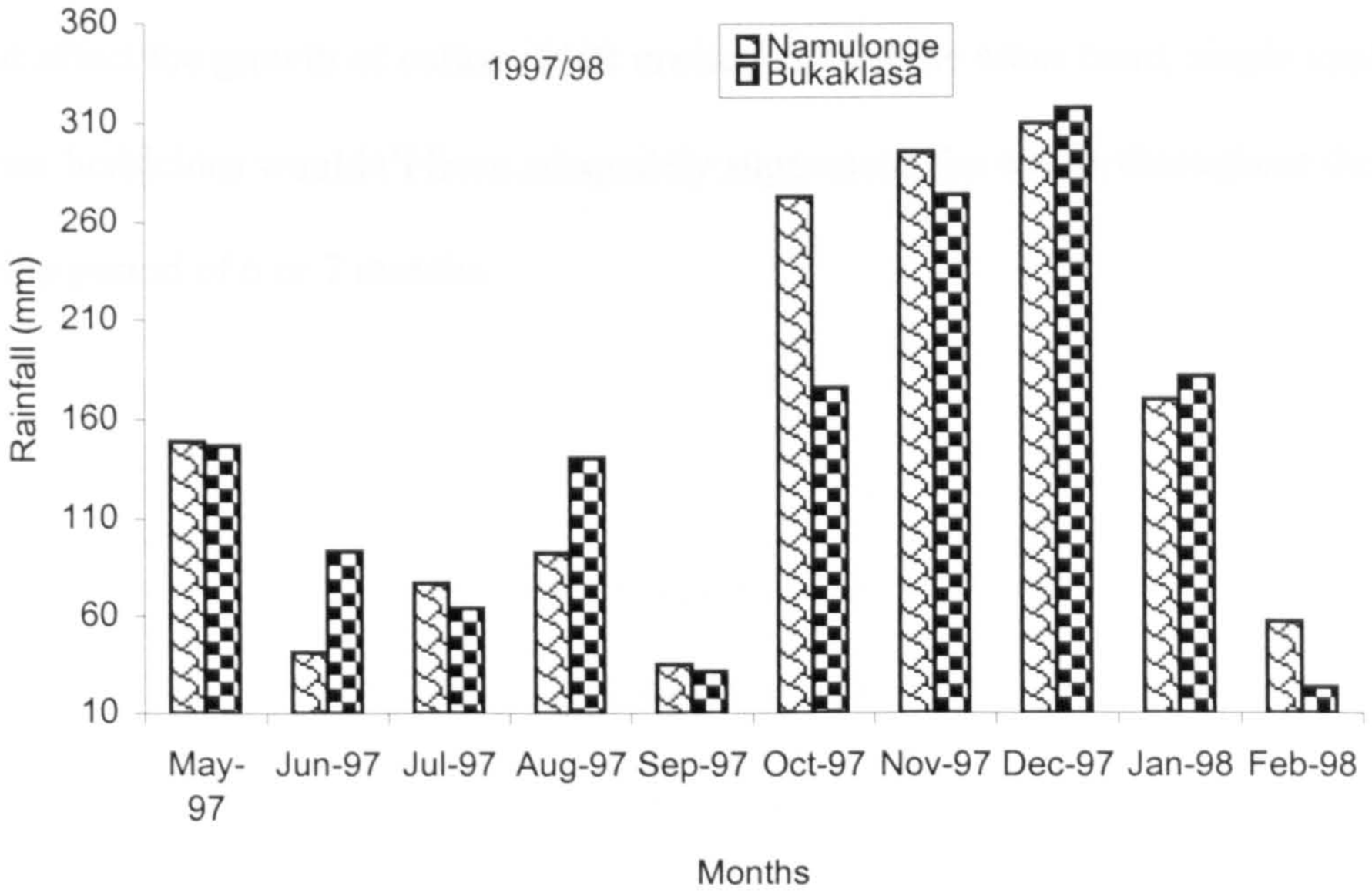


Figure 2.1. Mean monthly rainfall during the two cotton growing seasons for Namulonge and Bukalasa.

Two graminicide post-emergence herbicides, sethoxydim (checkmate) emulsifiable concentrates (193 g/L, EC) (Rhône Poulenc Agrochemical Co.,) and fluazifop-butyl (fusilade) emulsifiable concentrates (125 g/L, EC) (Zeneca Agrochemical Co.,) were sprayed on the perennial weed *Digitaria abyssinica* at its full ground cover in a pre-flowering growth stage. The herbicides were sprayed at dose rates recommended by the manufacturer and the reduced levels. Other common annual grass weeds identified in the field included *Panicum maximum*, *Sorghum halepense*, *Cynodon dactylon*, *Digitaria velutina*, *Elusine indica* were treated at the same time with *D. abyssinica.* Combinations of herbicide dose rates and hand weeding were done in this study. This was due to the fact that the herbicides used are grass selective and as a result the broadleaf weed species would affect the growth of cotton if left unchecked. On the other hand, single application of these herbicides wouldn't have adequately suppressed the weeds throughout the cotton growing period of 6 or 7 months.

Table 2.1 Herbicides evaluated for the control of *D. abyssinica* at reduced dose rates combined with two hand weeding supplements.

Treatment	Dose rates (g a.i.ha^{-1})	Proportion of full rate (%)
Fluazifop-butyl (Fusilade) + 2 hand weedings	138	70
Fluazifop-butyl (Fusilade) + 2 hand weedings	162	85
Fluazifop-butyl (Fusilade) + 2 hand weedings	188	100
Sethoxydim (Checkmate) + 2 hand weedings	405	70
Sethoxydim (Checkmate) + 2 hand weedings	502	85
Sethoxydim (Checkmate) + 2 hand weedings	579	100
Hand weeding	5 times	0
Control (untreated)	0	0

The above dose rates were obtained by reducing the full rates of each herbicide by 15 and 30%.

2.2.3. Crop and weed species

The cotton variety BPA 95 used in the study is mainly grown in the Central and Western regions in Uganda. This variety is rainfed, and it is an improved cotton variety which has inherent tolerance to some diseases such as bacterial blight (*Xanthomonas malvacearum*) which is caused by *Xanthomonas Camptris* Pv *malvacearum*. This variety was sown on different experimental fields each season at Namulonge and Bukalasa. The fields used during the two seasons are part of the experimental area of these two sites. In each site, the experimental fields were selected on grounds that they were heavily infested with *D. abyssinica* and that there were no previous weed control trials conducted at least for the

last 5 years, since this might affect the results. Details of the crops which were previously sown on these experimental fields are given in section 2.3. All experiments were laid out in such away that the rows were running against the contour in each field to avoid any form of erosion. The experimental fields layout is illustrated in Appendix 2.13. Land preparation involved two ploughings (25 cm depth) and one disc harrowing. No fertilizers were applied during seedbed preparation nor to the established crop. After seedbed preparation but prior to planting, soil sampling was done to the depth of 30cm, using soil auger. Soil sampling was done on all experimental fields of both sites for the two seasons. The soil samples were taken on four lines across the field, with five samples taken on each line. Each experimental field was divided in such away that the lines crossed the field diagonally, one line longitudinally and other line crossed the field transversely (forming a star pattern). The soil samples obtained from each line were mixed together to make one sample for an individual field prior to nutrient analysis for each experimental plot. For the two seasons soil samples were taken from different experimental fields at both sites. The soil nutrient analysis was done at Makerere University Kampala, Uganda (Table 2.2). Methods of soil nutrient analysis were done as described by Rowell, (1994), Ca, Mg, K, and Na (ammonium acetate method); Organic matter (Walkley and Black digestion); Available P (Bray I procedures); Total N (Kjeldhal method); silt, sand and clay (pipette method and USDA particle size fractions).

Table 2.2. Analysis of soil samples collected from Namulonge and Bukalasa for the two seasons.

Dry soil sample analysis	Namulonge 1995/96	Bukalasa 1995/96	Namulonge 1997/98	Bukalasa 1997/98
pH	6.0	5.5	5.9	5.3
O.M (%)	2.99	2.14	2.24	3.15
Available PO4 (p.p.m)	12.2	0.9	8.25	6.05
Total N (%)	0.08	0.02	0.18	0.15
Exchangeable Na (me/100g)	0.17	0.17	0.09	0.09
Exchangeable Ca (me/100g)	6.6	7.5	4.0	4.0
Exchangeable K (me/100g)	0.91	0.59	1.05	0.46
Exchangeable Mg (me/100g)	2.38	1.87	1.29	2.80
Sand (%)	57	68	68	59.0
Clay (%)	22.0	14	21	28.0
Silt (%)	27.0	18.0	11.0	13.0
Textural class	Loam	S/Loam	Loam	S/Loam

% – Percentage, O.M – Organic matter, S/Loam – Sandy loam

2.2.4. Experimental procedure during the two seasons

The field experimental procedures were the same for the two seasons and sites. Variety BPA 95 was sown in 5 m x 10 m plots at a spacing of 70cm x 50cm. Cotton seeds were sown on the 21/8/1995 at Namulonge while at Bukalasa sowing was done on the 22/8/195. During the 1997/98 season, sowing was done on the 12th and 14th /8/1997 at Namulonge and Bukalasa respectively. The sowing of cotton was somehow delayed during the 1995/96 season compared to 1997/98 season. This was due to less moisture in 1995/96 in the month of August, as it is illustrated in Figure 2.1. For both seasons sowing was done by hand, using a long chain on which the spacings were already marked. Holes were done following the markings on the chain, and they were made using hoes. Fourteen days after the emergence of cotton and weeds, the herbicides were sprayed. Spraying of sethoxydim and fluazifop-butyl was carried out after taking water volume calibrations. During calibrations, walking speed was about 1m. s $^{-1}$. A CP15 hand sprayer fitted with flat fan nozzle and operated at low pressure of 1 bar (100 kPa) was used. The hand sprayer was used to apply the herbicides at water volume rate of 12 L for the 4 plots of each dose rates, (600 L ha^{-1}). The application of the herbicides was done 14 days after the emergence of the cotton and weeds. Incidences of cotton insect pests such as cotton lygus were observed in the experimental fields at 35 days after planting. They were sprayed with Ripcord cypermethrin 6% EC) (Rhône Poulenc Agrochemical Co.,). Spraying of Ripcord was done at 1.0 L ha^{-1} in 12 L of water using CP15 hand sprayer. Spraying was done four times commencing from 35 days after planting and subsequent three sprayings were done at two week interval. Diseases such as bacterial blight, *Ramularia areola* and *Verticillium dahlia* were observed on the cotton plants but they were not of economic importance.

2.2.5. Experimental measurements

2.2.5.1 Weed counts

Weeds in the fields, mainly the grass species were sampled and counted before treatment and 35 days after herbicide application. Various weed species identified were *D. abyssinica, Panicum maximum, Sorghum halepense, Cynodon dactylon, Eleusine indica, Digitaria velutina, Cyperus* species, *Galinsoga parviflora, Oxalis latifolia, Bidens pilosa, Amaranthus* species and others which consisted of unidentified broadleaf and grass weeds. Weed count was done for *D. abyssinica*, *P. maximum* and *C. dactylon* because of their high densities, in addition *Sorghum halepense* was also counted as there was a heavy infestation in the 1997/98 season. Weeds were sampled in 30 cm x 30 cm (0.3 m x 0.3 m) quadrats which were randomly thrown four times in each plot. Group of shoots of *D.abyssinica* (the above ground foliage) rising from one node were counted as a single plant. Estimation of herbicide weed control was done. The number of weeds counted from the treated plots was subtracted from those counted from the control plots and was expressed as a percentage of the total number of the weeds counted from the control. The two supplementary hand weedings were done after weed counts (35 days after treatment), and they were done at 3 week intervals. The hand weeded plots were weeded five times at 3 week intervals after each weeding. The weeding was started at fourteen days after the emergence of cotton and weeds. Hand weeding in cotton usually starts 10-14 days after the emergence of the crop and the weeds. It can however be done earlier or later than that depending on the weed density or sometimes the weather conditions. Most weed species tend to grow fast in wet

conditions compared to dry conditions, in this case weeding can even start earlier than fourteen days.

2.2.5.2. Measurement of fresh and dry weights of *D. abyssinica*

After the weed counts, the shoots of *D. abyssinica* were harvested from the four quadrats in each plot. The harvested shoots were placed in paper bags and weighed to determine the fresh weight. During the 1995/96 season dry weight of *D. abyssinica* shoots was not determined due to lack of facilities. However, during the 1997/98 season both fresh and dry weights of *D. abyssinica* were determined. All weights were determined using a balance GT 800 (Ohaus Corp-Florham park).

2.2.5.3. Measurement of crop performance

The effect of treatments on the crop performance was evaluated by taking measurements on the cotton plant stand from individual plots. Each plot contained eight rows but the measurements were recorded from the four middle rows to avoid variations which could have been caused by the treatments from the neighbouring plots. Measurements included plant height, number of sympodia (fruiting branches), number of bolls per plant and yields. Data recording of these parameters started at 90 days after cotton planting (90DAP). The data were recorded from ten plants randomly selected from the four internal rows of each plot. This continued at a 15 day interval up to crop maturity (approximately 165 DAP). Cotton was harvested from the four internal rows in each plot. Cotton was hand picked and this was done three times at 2 week intervals. Sometimes harvesting frequency may depend on the weather conditions because during the wet conditions the opening of

mature bolls tends to take longer than during the dry period. Seedcotton was picked from each plot and placed in hessian bags. The hessian bags were marked according to plot numbers. Seedcotton from the three harvests of each plot was mixed and weighed to determine the weight of seedcotton per unit area harvested ($21m^2$).

2.2.5.4. Data analysis

Data collected on the percentage weed control, fresh and dry weights and crop performance were subjected to analysis of variance (ANOVA). The means were separated at 5% level significance using Tukey's multiple range test. Data obtained in percentages was transformed prior to ANOVA using ArcSine $\sqrt{}$ percentage transformation.

2.3. Results and Discussion

In the two seasons of 1995/96 and 1997/98, the experimental fields used in the study were mainly heavily infested with *D. abyssinica* although other grass weed species were present. During the 1995/96 season, cotton was sown in the field which previously had a sweet potato (*Ipomaea batatas*) crop at Namulonge, while at Bukalasa the previous crop in the experimental field was an inter-crop of ground nuts (*Arachis hypogaea*) and maize (*Zea mays*). In the 1997/98 season cotton sowing was done in fields which previously had pure stands of maize (*Zea mays*) at Namulonge and beans (*Phaselous vulgaris*) at Bukalasa. During both seasons, all field operations (ploughing, sowing, spraying harvesting) were timely done. The weather conditions was fine on the days the herbicides were sprayed, although in 1997/98 it rained later in the day at Namulonge, however, the rains never

affected the performance of the herbicides. All the experiments were successfully conducted during the two seasons for both sites.

2.3.1. Percentage weed control

The two field trials conducted in the 1995/96 and 1997/98 seasons revealed that the use of sethoxydim and fluazifop-butyl followed by two supplementary hand weedings improved the spectrum of weed control in cotton compared to the control. Concerning the percentage weed control of *D. abyssinica* in cotton fields, it was noted that sethoxydim and fluazifop-butyl significantly reduced *D. abyssinica* in the two sites for two seasons (Table 2.3). No significant differences were noted in the control of *D. abyssinica* amongst dose rates of each of the two herbicides during the two seasons at the two sites. The high percentage weed control obtained in this study indicated that there was almost total control of the foliage of *D. abyssinica* at 35 days after herbicide application from all the dosage rates of both herbicides. Good weed control of *D. abyssinica* with sethoxydim has been reported elsewhere (Parker, 1982). In contrast however, Parker 1982, reported low activity of fluazifop-butyl against *D. abyssinica.* Research elsewhere has also revealed the high activity of sethoxydim and fluazifop-butyl against other grass species such as alexandergrass (*Brachiaria plantaginea*) (Fleck, 1994). The results in the present study have also revealed that these post emergence grass selective herbicides also controlled *Cynodon dactylon*, *Panicum maximum* and *Sorghum halepense* (Appendices 2.1 and 2.2). This supported results obtained elsewhere on the control of various grass weed species with sethoxydim and fluazifop-butyl (Harker and O'Sullivan, 1991; Perry, 1994).

The present results however, suggested that the activity of the reduced dose rates (138, 162 and 405, 502 g a.i.ha^{-1} of fluazifop-butyl and sethoxydim respectively) against *D. abyssinica* was as good as the full dose rates (fluazifop-butyl, 188 g a.i.ha^{-1} and sethoxydim, 579 g a.i.ha^{-1}). This is illustrated in the scatter graphs (Appendices 2.3 and 2.4), where percentage control was not associated with herbicide concentrations. In a field visual observation, it was noted that the control of grasses gave favourable conditions for vigorous growth of the broadleaf weeds (Figure 2.2). These included *Oxalis latifolia*, *Bidens pilosa, Galinsoga parviflora, Portulaca oleraceae*, *Solanum nigrum*, *Ageratum conyzoides* and others. These broadleaf weed species are not difficult to control. They were suppressed by the two supplemented hand weedings.

Table 2.3. Percentage weed control of *D. abyssinica* obtained after herbicide application in two sites for two seasons in the field.

Treatment	**Namulonge 1995/96**	**Bukalasa 1995/96**	**Namulong 1997/98**	**Bukalasa 1997/98**
Fluazifop-butyl 138 g a.i.ha^{-1}	79.5±3.6a	82.5±3.9a	82.5±2.1a	87.8±2.4a
Fluazifop-butyl 162 g a.i.ha^{-1}	91.3±4.7a	87.3±3.5a	91.5±2.5a	91.0±1.7a
Fluazifop-butyl 188 g a.i.ha^{-1}	86.0±2.2a	90.8±1.4a	91.3±1.3a	92.8±1.1a
Sethoxydim 405 g a.i.ha^{-1}	83.8±2.7a	81.3±3.8a	85.0±1.8a	92.0±0.4a
Sethoxydim 502 g a.i ha^{-1}	96.0±0.7a	90.0±2.7a	89.0±1.9a	92.5±1.3a
Sethoxydim 579 g a.i ha^{-1}	86.0±3.0a	93.8±2.0a	84.8±2.7a	89.8±2.7a
Significance level	ns	ns	ns	ns

ns - not significant

± - represents standard error for each mean value of four replications

Figure 2.2. Broadleaf weeds grow vigorously after the control of grass weed species in the field.

2.3.2. Percentage reduction of fresh and dry weight of *D. abyssinica* after herbicide application

Control of *D. abyssinica* was also assessed by using percentage reduction of fresh and dry weight of the *Digitaria* shoots (foliage). Results from the two field trials of the two seasons indicated that foliar applications of fluazifop-butyl (138, 162 and 188 g a.i ha^{-1}) and sethoxydim (405, 502 and 579 g a.i.ha^{-1}) significantly reduced the fresh and dry weight of *D. abyssinica* (Figures 2.3 and 2.4). However, during the 1995/96 season, dry weight of *D. abyssinica* was not assessed. The significant reduction of the fresh and dry weight of *D. abyssinica* during the two seasons can confirm growth inhibition of this weed following the application of fluazifop-butyl and sethoxydim. Growth inhibition of other grass species due to the application of sethoxydim and fluazifop-butyl has been reported elsewhere (Jain and Vanden-Born, 1989). The present results showed that the reduction of fresh weight obtained in the two sites during the two seasons did not significantly differ amongst dose rates of both herbicides. There was no dose response observed, probably suggesting the adequate activity of low dose rates against *D. abyssinica*. A similar trend was almost observed in the reduction of dry weight in the 1997/98 season, although the lowest dose rate of fluazifop-butyl (138 g a.i.ha^{-1}) significantly differed from doses 162 and 188 g a.i.ha^{-1} at Namulonge. However, the percentage reduction of dry weight obtained from this lowest dose rate was still high for efficient control of the weed especially when combined with hand weeding.

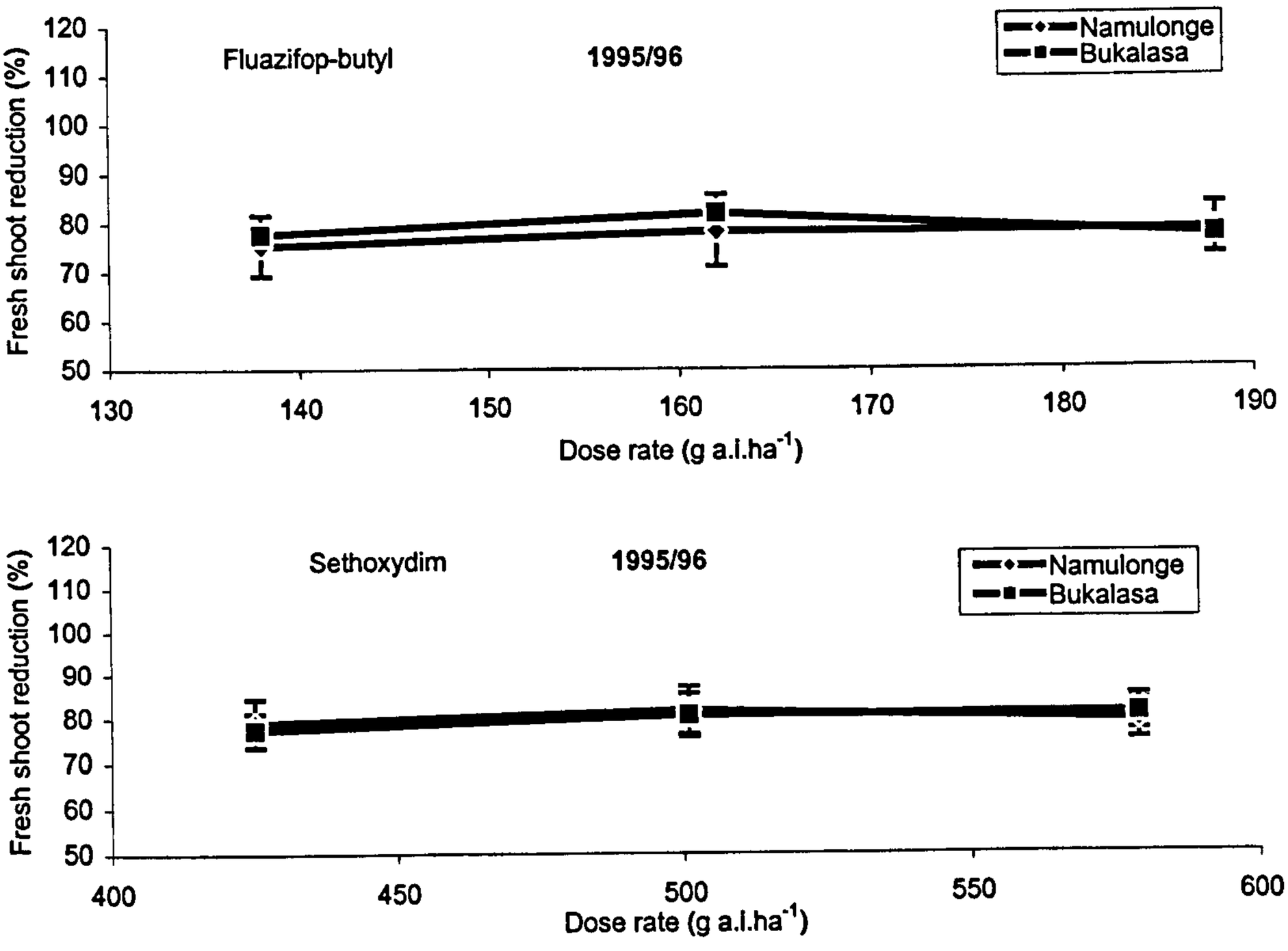
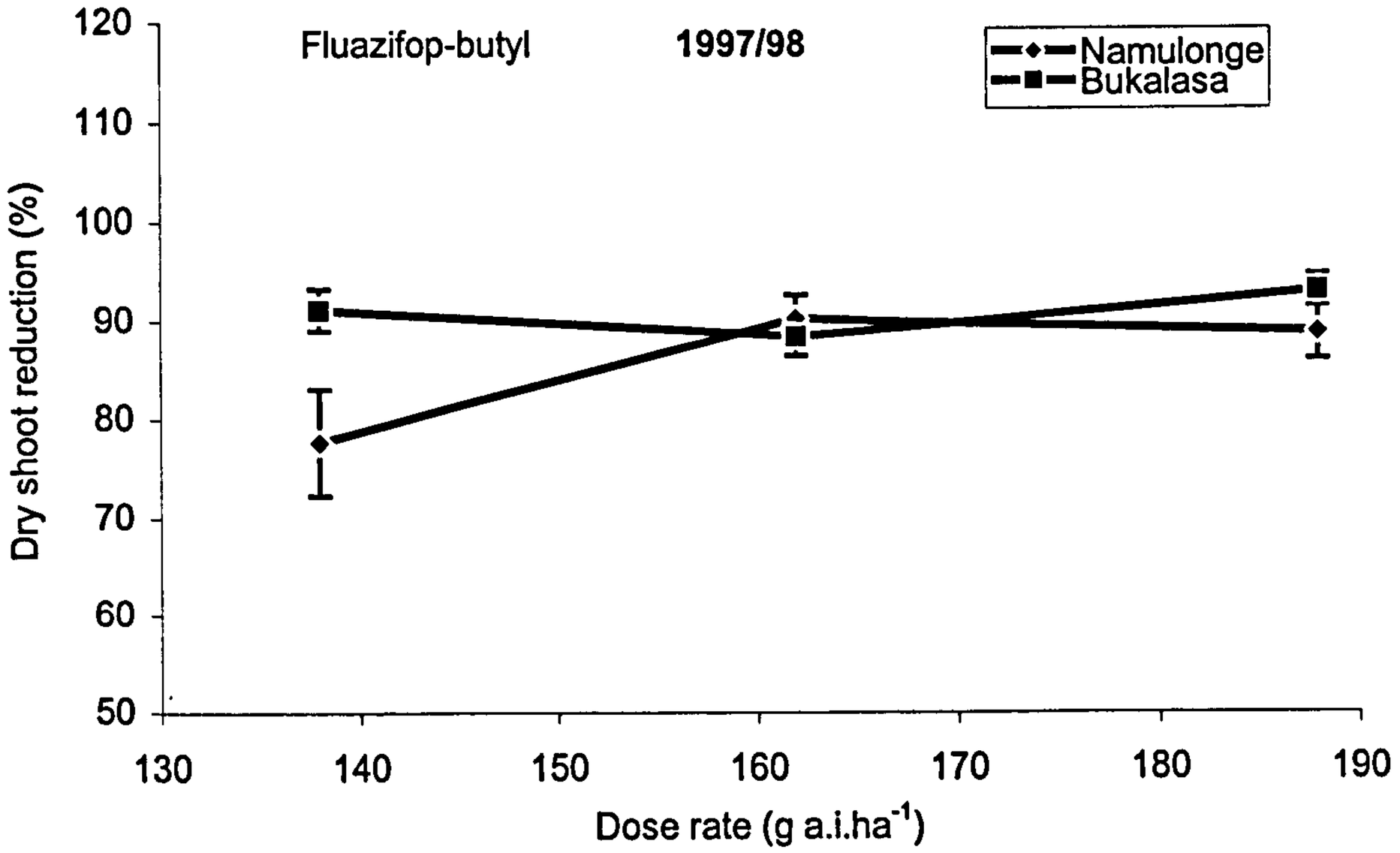


Figure 2.3. Percentage reduction of fresh weight of *D. abyssinica* shoots determined after herbicide application in two sites for two seasons. in the field.
Bars represent standard error of each mean value of four replications.



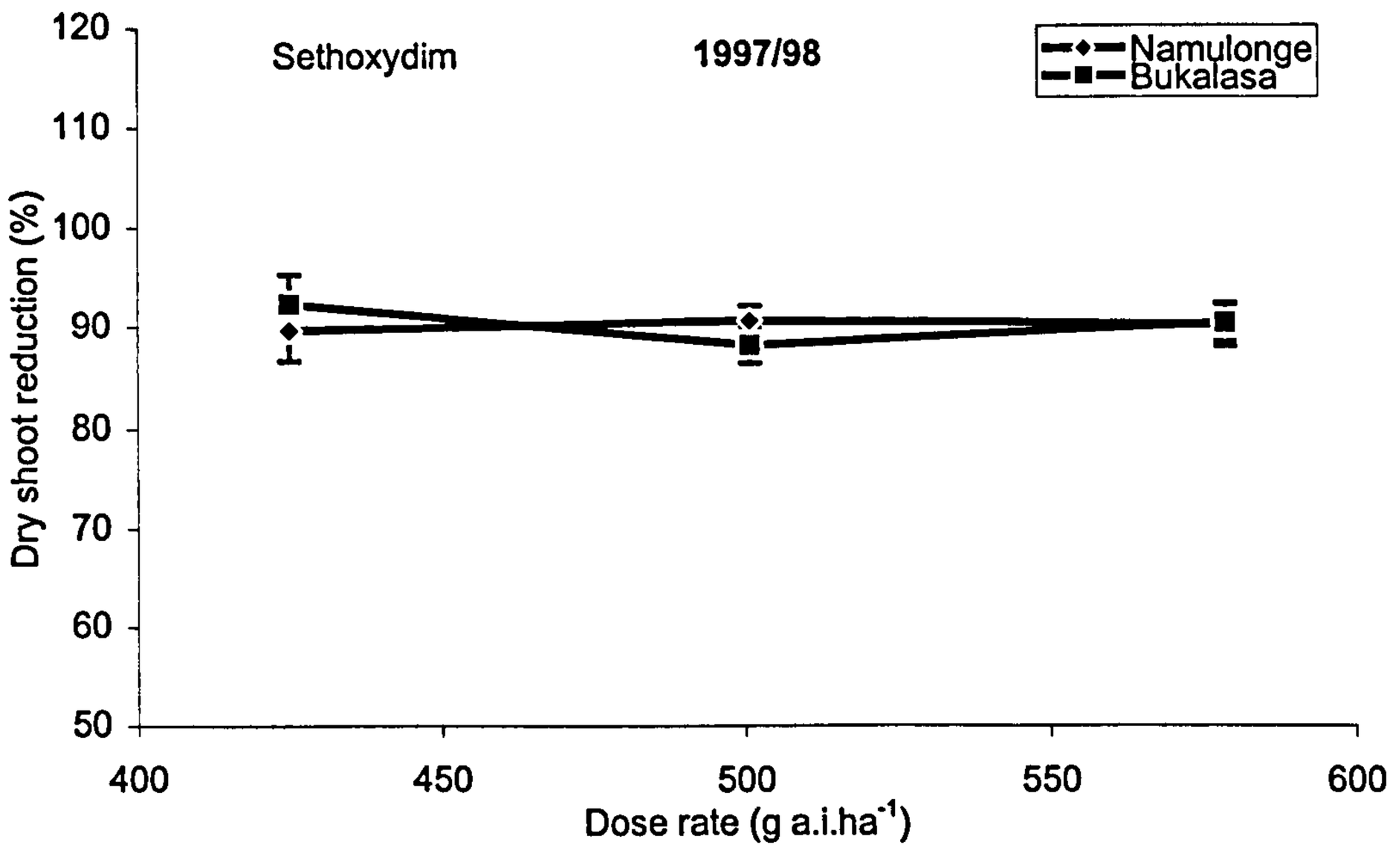


Figure 2.4. Percentage reduction of dry weight of *D. abyssinica* shoots determined after herbicide treatment in two sites for one season in the field.
Bars represent the standard error of each mean value of four replications.

The average percentage reduction of both fresh and dry weight of *D. abyssinica* shoots was between 70 and 80% irrespective of dose rates at the two sites during the 1995/96 and 1997/98 seasons. This observation supported previous research which indicated reduction of fresh weight other perennial grass weed species such as *Elymus repens* following the application of sethoxydim and fluazifop-butyl (Hicks and Jordan, 1983).

2.3.3. Crop performance

2.3.3.1. Plant height and sympodia of cotton plants

The number of sympodial branches and plant height do reflect the growth vigour of a cotton plant which might indicate the availability of nutrients. But when weeding is not done or delayed, this may result in plant growth vigour reduction. Results obtained from the two seasons field trials indicated the significance of weed control on the cotton plant growth vigour (Tables 2.4 and 2.5). Analysis of variance showed that the control of weed species with sethoxydim and fluazifop-butyl combined with two hand weedings and the hand weeding (5 times), significantly increased the plant vigour compared to plants in the weedy plots. This was also noted in the data taken at different dates during the crop growth period (Appendices 2.5, 2.6, 2.7 and 2.8). The observation was noted in the two sites. These results supported research findings by Vencill *et al*, (1992) who also reported reduction of cotton plant height due to weed competition of cotton and coastal barmudagrass (*Cynodon dactylon*). Results presented in this study have indicated that there were no significant differences in the plant vigour obtained from the combination of herbicides and two hand weedings and weeding (5 times). Similarly the plant vigour obtained from the combination of dose rates

with the two hand weedings did not significantly differ from each other. However, further observation found that cotton plant height was high from plants obtained at Namulonge during the 1995/96 season while during the 1997/98 season the plant height was noted high in cotton plants obtained at Bukalasa. This variation seemed rather unexplainable, although it can generally be based on the availability of the soil nutrients of the different experimental fields used in these sites during the two seasons. And there could also be another possibility that the previous crops on these experimental fields were not heavy feeders and therefore the soils were not exhausted. Although analysis of variance indicated that there was no significant difference in plant height amongst treatments, the plant height response to weed control slightly varied amongst treatments possibly indicating weed-crop interactions. Despite the fluctuation in plant height between the two seasons, the number of sympodial branches was noted high in both sites in the trials of 1997/98 season compared to the 1995/96 trials.

Table 2.4. Mean cotton plant height (cm) measured at 165 days after planting in two sites for two seasons in the field.

Treatment	**Namulonge 1995/96**	**Bukalasa 1995/95**	**Namulonge 1997/98**	**Bukalasa 1997/98**
Fluazifop-butyl 138 g a.i.ha^{-1}+2hw	101.0±11.8a	98.0±5.0a	90.0±4.9a	112.8±7.7a
Fluazifop-butyl 162 g a.i.ha^{-1}+2hw	100.8±3.2a	94.8±5.1a	96.8±6.1a	110.3±9.3a
Fluazifop-butyl 188 g a.i.ha^{-1}+2hw	98.5±6.6a	91.0±5.3a	92.0±3.6a	116.5±10.4a
Sethoxydim 405 g a.i.ha^{-1}+2hw	101.0±4.2a	89.3±6.7a	94.8±5.5a	119.0±12.7a
Sethoxydim 502 g a.i.ha^{-1}+2hw	96.8±3.6a	93.3±9.0a	91.8±3.8a	108.5±10.6a
Sethoxydim 579 g a.i.ha^{-1}+2hw	100.3±7.9a	89.8±9.2a	101.0±8.5a	113.0±5.1a
Hand-hoe weeding (5 times)	106.0±7.9a	94.5±5.5a	99.0±2.0a	130.0±8.7a
Control	59.0±6.9b	61.3±5.3b	45.0±2.4b	62.3±2.3b
Significance level	**	**	**	**

** - highly significant at 1%, hw – hand weeding

± - represents the standard error of each mean value of four replications

Table 2.5. Mean sympodial branches per cotton plant counted at 165 days after planting in two sites for two seasons in the field.

Treatment	**Namulonge 1995/96**	**Bukalasa 1995/96**	**Namulonge 1997/98**	**Bukalasa 1997/98**
Fluazifop-butyl 138 g a.i.ha^{-1}+2hw	18.3±1.3a	16.3±1.7a	20.0±0.5a	21.5±0.9a
Fluazifop-butyl 162 g a.i.ha^{-1}+2hw	14.8±1.0a	17.0±1.2a	20.0±1.8a	26.0±1.7a
Fluazifop-butyl 188 g a.i.ha^{-1}+2hw	15.5±1.0a	17.0±0.8a	20.3±1.5a	25.5±1.8a
Sethoxydim 405 g a.i.ha^{-1}+2hw	17.3±1.0a	16.8±1.5a	21.5±1.6a	23.0±2.1a
Sethoxydim 502 g a.i.ha^{-1}+2hw	15.8±1.9a	14.0±1.4a	19.3±1.9a	21.8±2.1a
Sethoxydim 579 g a.i.ha^{-1}+2hw	17.3±2.2a	15.5±0.4a	20.5±1.8a	21.5±1.6a
Hand-hoe weeding (5 times)	16.5±2.7a	16.8±2.5a	20.5±2.2a	24.8±1.3a
Control	5.2±0.5b	6.0±0.8b	8.0±1.4b	8.0±2.7b
Significance leve	**	**	**	**

** - highly significant at 1%, hw – hand weeding
± - represents the standard error of each mean value of four replications.

2.3.3.2 Cotton yield components

The cotton yield components start with the flowers set on the sympodial branches. Flowering of cotton starts around 6 or 8 weeks after planting as flower buds or squares but it reaches its peak at 12 weeks (Serunjogi, 1994). A mature flower has 5 white/cream petals, which eventually turn to purple/pink with age and drops off leaving a young boll in the bracts. The bolls usually ripen between 50 to 70 days after fertilisation, but this might vary depending on the variety, temperature, humidity, flowering date and position of the bolls on the plant (Gipson and Ray, 1970).

Bolls reach maturation at about 140 days after cotton emergence or 84 days after flowering and when it dries at maturity the outer wall of each loculus opens, exposing the lint and the seed (seed cotton). Results obtained in this study showed that significantly high number of bolls per plant was obtained from plots where weeds were controlled (Table 2.6). This observation was noted at the two sites during the two seasons. There were no significant differences found in number of bolls per plant amongst the weed control treatments. On average however, the combination of herbicides dose rates with the 2 hand weedings gave high or equal number of bolls as obtained from the 5 times weeding treatment. Further observation found that the integrated treatments of fluazifop-butyl dose rates plus 2 hand weedings gave slightly high number of bolls compared to the sethoxydim dose rates supplemented with 2 hand weedings. This was observed at the two sites during the 1995/96 season, but in 1997/98 it was the sethoxydim combinations that gave higher numbers of bolls. The differences amongst these treatments were not significant, suggesting good weed control with reduced dose rates of both herbicides combined with hand weeding in cotton. This is also illustrated in the number of bolls obtained at different dates during plant growth in the two seasons at the two sites (Appendices 2.9 and 2.10). Generally however, the number of bolls per plant obtained at 165 days after planting was high during the 1995/96 season compared to the 1997/98 season at the sites. A similar observation was found in the control plots irrespective of high weed infestation. The number of bolls per plant during the 1997/98 season could have been affected by the excessive rains during boll-formation (rainfall data, Figure 2.1). Akobundu, (1987c) has clearly explained the effect of water distribution during boll-formation and boll-ripening in cotton.

Table 2.6. Mean number of bolls per cotton plant obtained at 165 dap in two sites for two seasons in the field.

Treatment	**Namulonge 1995/96**	**Bukalasa 1995/96**	**Namulonge 1997/98**	**Bukalasa 1997/98**
Fluazifop-butyl 138 g a.i.ha^{-1}+2hw	12.0±0.9a	12.5±1.1a	8.5±0.3a	10.0±0.8a
Fluazifop-butyl 162 g a.i.ha^{-1}+2hw	13.8±1.2a	12.0±0.8a	10.8±0.9a	11.3±0.6a
Fluazifop-butyl 188 g a.i.ha^{-1}+2hw	11.8±1.0a	10.8±0.5a	9.3±0.6a	11.5±0.6a
Sethoxydim 405 g a.i.ha^{-1}+2hw	11.5±1.1a	10.3±0.9a	10.0±0.8a	11.5±0.9a
Sethoxydim 502 g a.i.ha^{-1}+2hw	12.5±1.4a	7.8±0.5a	11.8±0.9a	11.3±0.5a
Sethoxydim 579 g a.i.ha^{-1}+2hw	11.8±1.0a	10.5±0.5a	9.3±0.5a	10.8±0.3a
Hand-hoe weeding (5 times)	13.3±1.3a	11.8±0.8a	9.0±0.0a	10.8±0.5a
Control	4.0±0.3b	3.5±0.3b	1.3±0.3b	2.3±0.3b
Significance level	**	**	**	**

** - highly significant at 1%, hw – hand weeding
± - represents the standard error of each mean value of four replications

Seedcotton yields realised from the two seasons are presented in Table 2.7. Results revealed that although the combinations of 2 hand weedings with fluazifop-butyl dose rates had slightly high number of bolls per plant, high seed cotton yields were obtained from the combinations of sethoxydim dose rates with the 2 hand weedings. This observation was noted in 1995/96 at the two sites. This possibly suggested that the number of bolls per plant did not influence the seed cotton yields. According to Hake *et al.*, 1990 the size and the weight of the bolls at maturation may limit fibre development. In another observation, the present results indicated that cotton sowed in 1995/96 yielded.

Table 2.7. Seedcotton yields (kg ha^{-1}) obtained from the cotton fields at Namulonge and Bukalasa for two seasons.

Treatement	Namulonge 1995/96	Bukalasa 1995/96	Namulonge 1997/98	Bukalasa 1997/98
Fluazifop-butyl 138 g $a.i.ha^{-1}$+2hw	1962.5±209.3a	1793.7±156.3a	665.1±67.7a	891.1±147.9a
Fluazifop-butyl 162 g $a.i.ha^{-1}$+2hw	2993.7±52.4a	2368.8±231.0a	915.5±98.5a	1184.2±108.9a
Fluazifop-butyl 188 g $a.i.ha^{-1}$+2hw	2706.2±48.3a	2181.2±151.9a	908.6±95.3a	880.6±56.1a
Sethoxydim 425 g $a.i.ha^{-1}$+2hw	2325.0±283.9a	2100.0±245.8a	704.1±74.8a	745.5±187.9a
Sethoxydim 501 g $a.i.ha^{-1}$+2hw	2887.5±227.4a	2237.5±179.6a	1002.5±80.5a	859.3±89.1a
Sethoxydim 579 g $a.i.ha^{-1}$+2hw	2675.0±264.8a	2112.5±42.7a	1007.1±73.7a	941.8±157.9a
Hand weeding (5 times)	2681.2±224.6a	2475.0±185.5a	898.8±92.8a	919.8±71.5a
Control	43.0±21.4b	47.5±31.0b	31.6±13.5b	47.6±17.1b
Significance level	**	**	**	**

** - highly significant at 1%, hw – hand weeding
± - represents standard error of each mean value of four replications

highly compared to the cotton sowed in 1997/98. This may again be associated with the excessive rains obtained during this season as earlier on mentioned. However, the analysis of variance showed that there was no significant difference in yields amongst all the weed control treatments in each season at the two sites. These results indicated the role of 2 hand weeding supplememnts combined with the low levels of fluazifop-butyl and sethoxydim for the control of *D. abyssinica* and other weed species in cotton production. At the same time results suggested that weed management integration can reduce the number of weedings and improve cotton yields. Research elsewhere reported increase of seed cotton yields following the control of various grass weed species with sethoxydim and fluazifop-butyl in cotton (Makhan'kova *et al.*, 1987;Byrd *et al.*, 1987). In other studies supplementary weed control in addition to herbicide treatments in cotton production has been emphasised (Lagoke and Choudhary, 1982; Lagoke *et al.*, 1986; Adigun, 1984; Bakut, 1985; Lagoke *et al.*, 1992). On the other hand, the present results revealed that cotton-weed competition for a full season markedly reduced seed cotton yields during the two seasons at both sites. Yield reduction in the weedy plots was estimated between 80-90% compared to the yields obtained from the weed control plots. These results agreed with the research findings by Patterson *et al.*, (1979); Keeley *et al.*, (1987); Snipes and Mueller, (1992) who observed high cotton yield losses due to heavy weed infestation.

2.3.3.3. Economic analysis of weed control treatments/methods in cotton

The study was initiated to investigate various weed management treatments which could reduce the need weeding and thus save labour. The economics of the treatments used in the study are illustrated in Figures 2.5 and 2.6, based on experimental results for different weed control treatments from Namulonge and Bukalasa stations in two different seasons.

In these figures, numbers 1-7 represent the treatments as follows;

1) Fluazifop-butyl 138 g a.ha^{-1} (1.1L) + 2 hand weedings

2) Fluazifop-butyl 162 g a.i.ha^{-1} (1.3L) + 2 hand weedings

3) Fluazifop-butyl 188 g a.i.ha^{-1} (1.5L) + 2 hand weedings

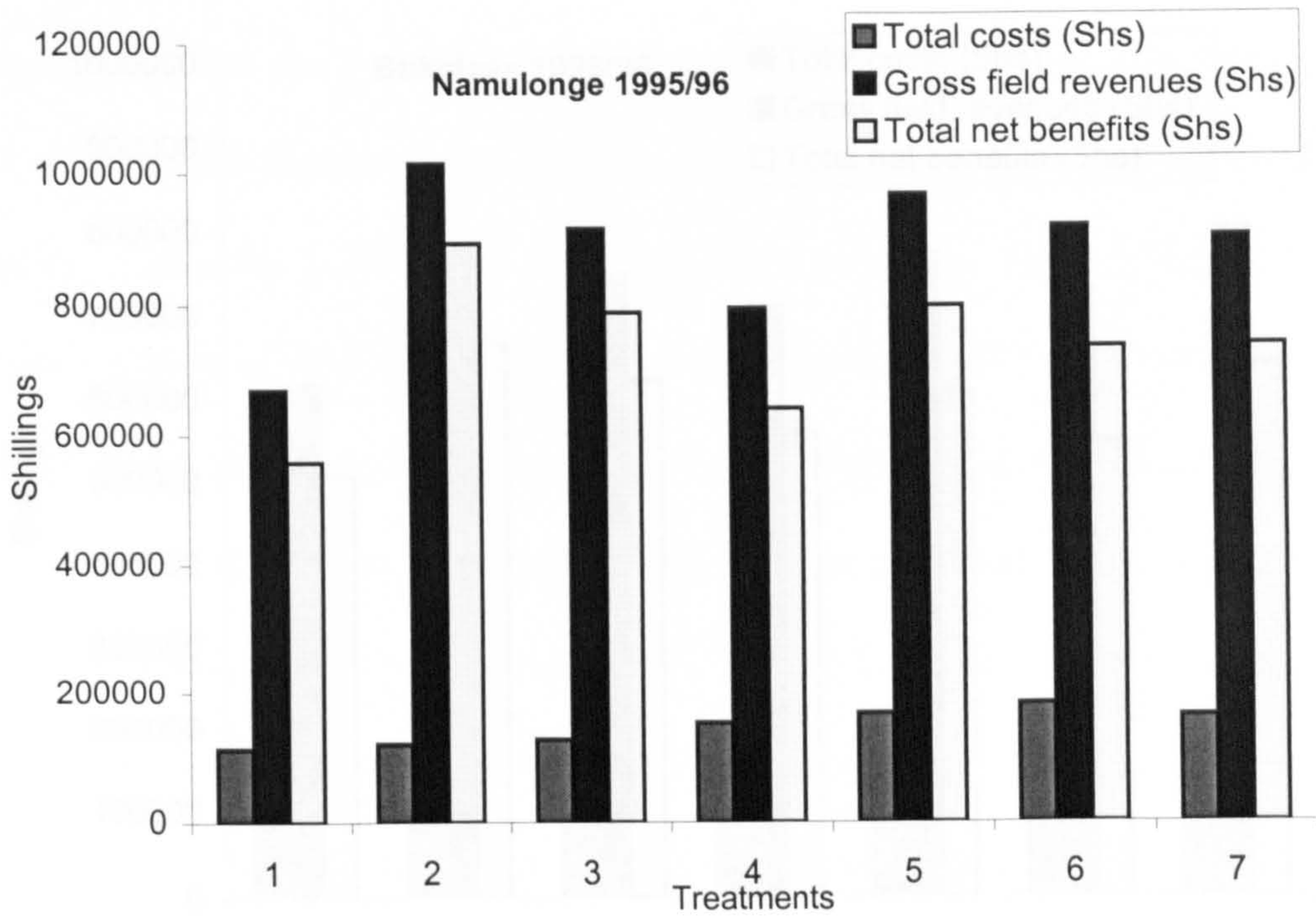
4) Sethoxydim 405 g a.i.ha^{-1} (2.2L) + 2 hand weedings

5) Sethoxydim 502 g a.i.ha^{-1} (2.6L) + 2 hand weedings

6) Sethoxydim 579 g a.i.ha^{-1} (3.0L) + 2 hand weedings

7) Hand-hoe weeding (5 times)

The seedcotton yield data as well as the inputs used in the experiments were used for economic analysis (revenues and costs), the values are illustrated in Appendices 2.11 and 2.12. The gross margins were thus calculated on the basis of the yield and quality mix results already reported, using a labour cost of shs. 1500 per day, with 3 days required for spraying and 22 days required for each weeding time. The chemical costs are those charged for the applications of each spray, and shs. 500 is charged for hire of the hand sprayer CP15 (Appendices 2.11 and 2.12). The results of these illustrative calculations are shown in Figures 2.5 and 2.6 for each treatment, and Figures 2.7 and 2.8 for the averages



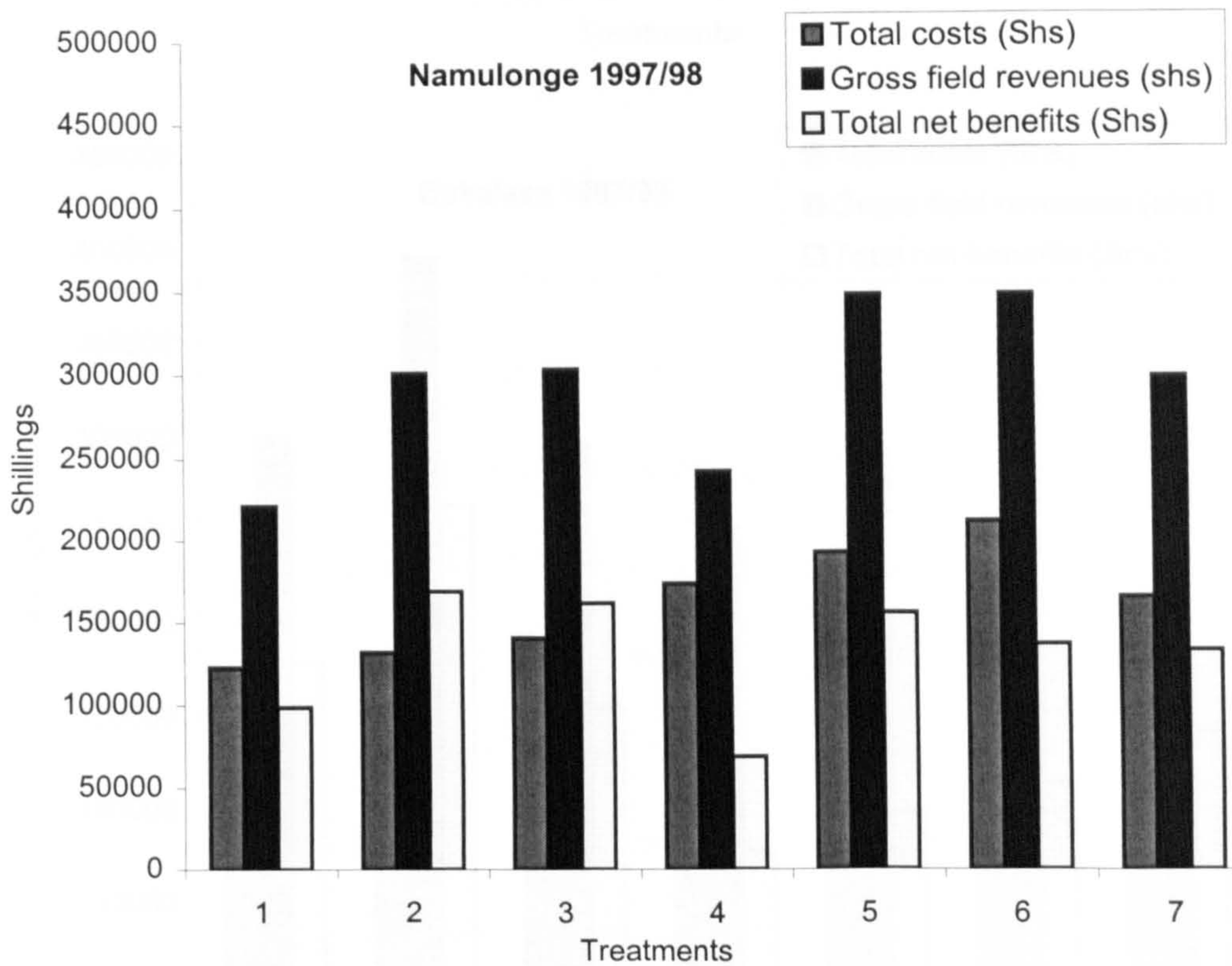
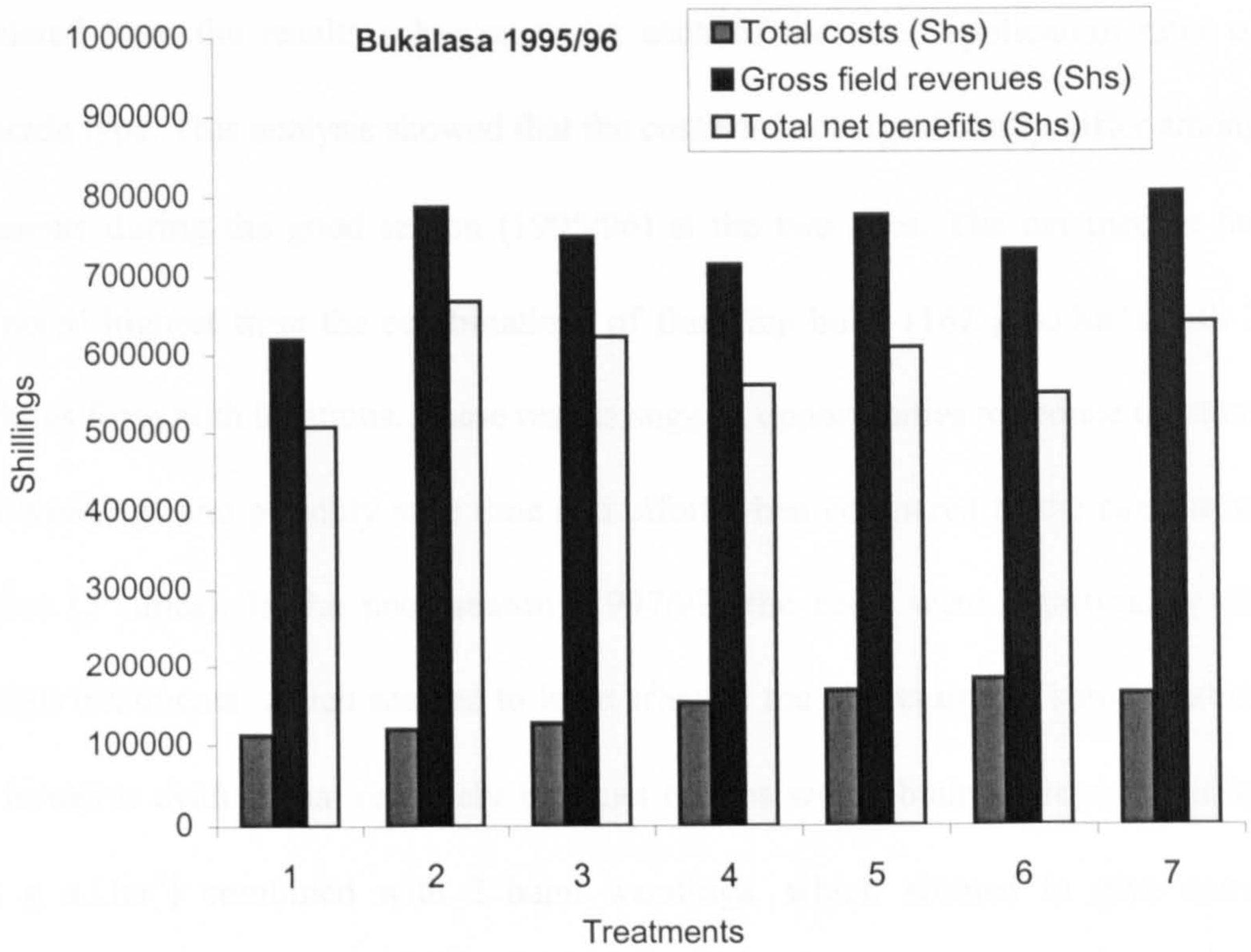


Figure 2.5 Effects of the weed control treatments on the economics of cotton production per hectare at Namulonge.



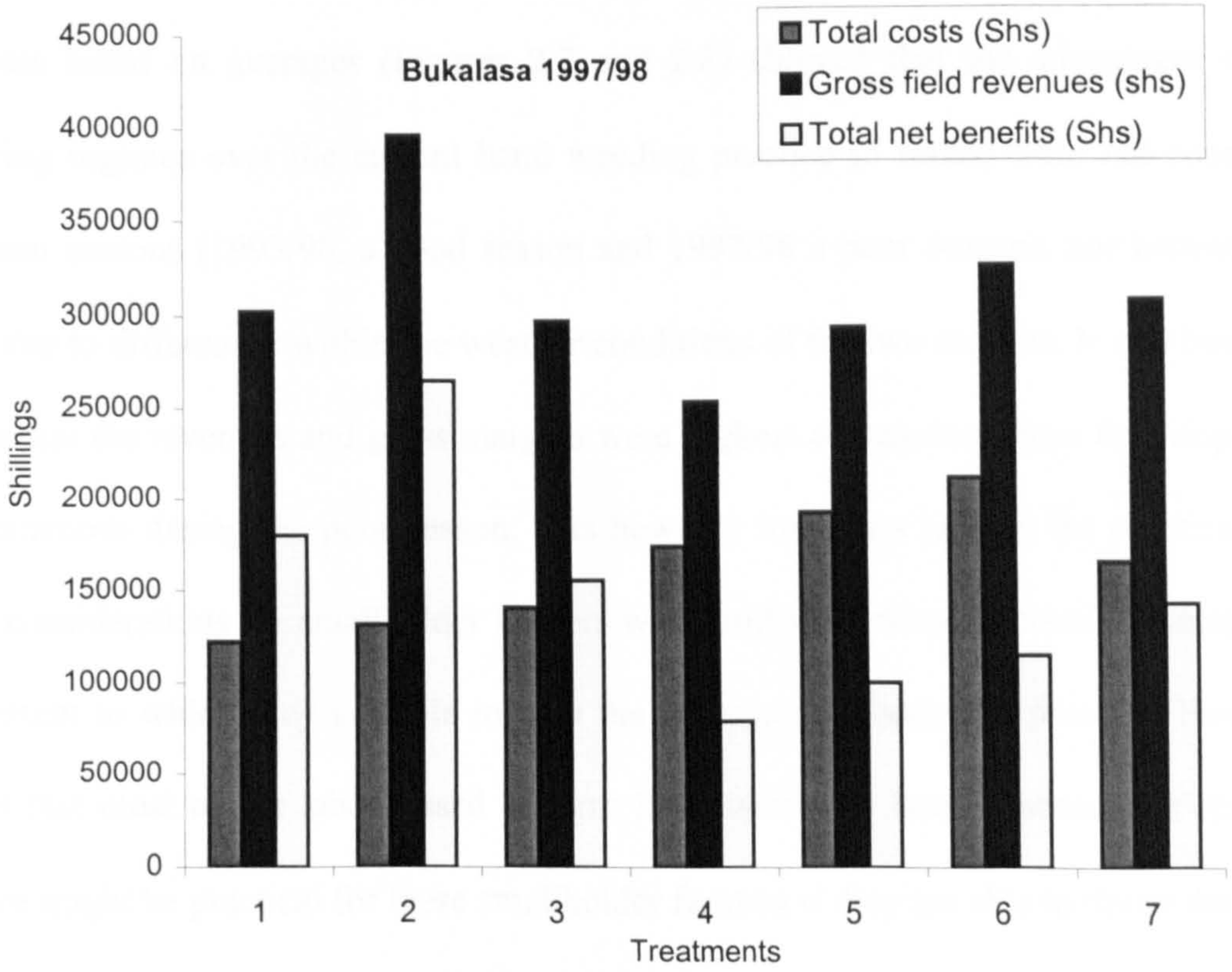
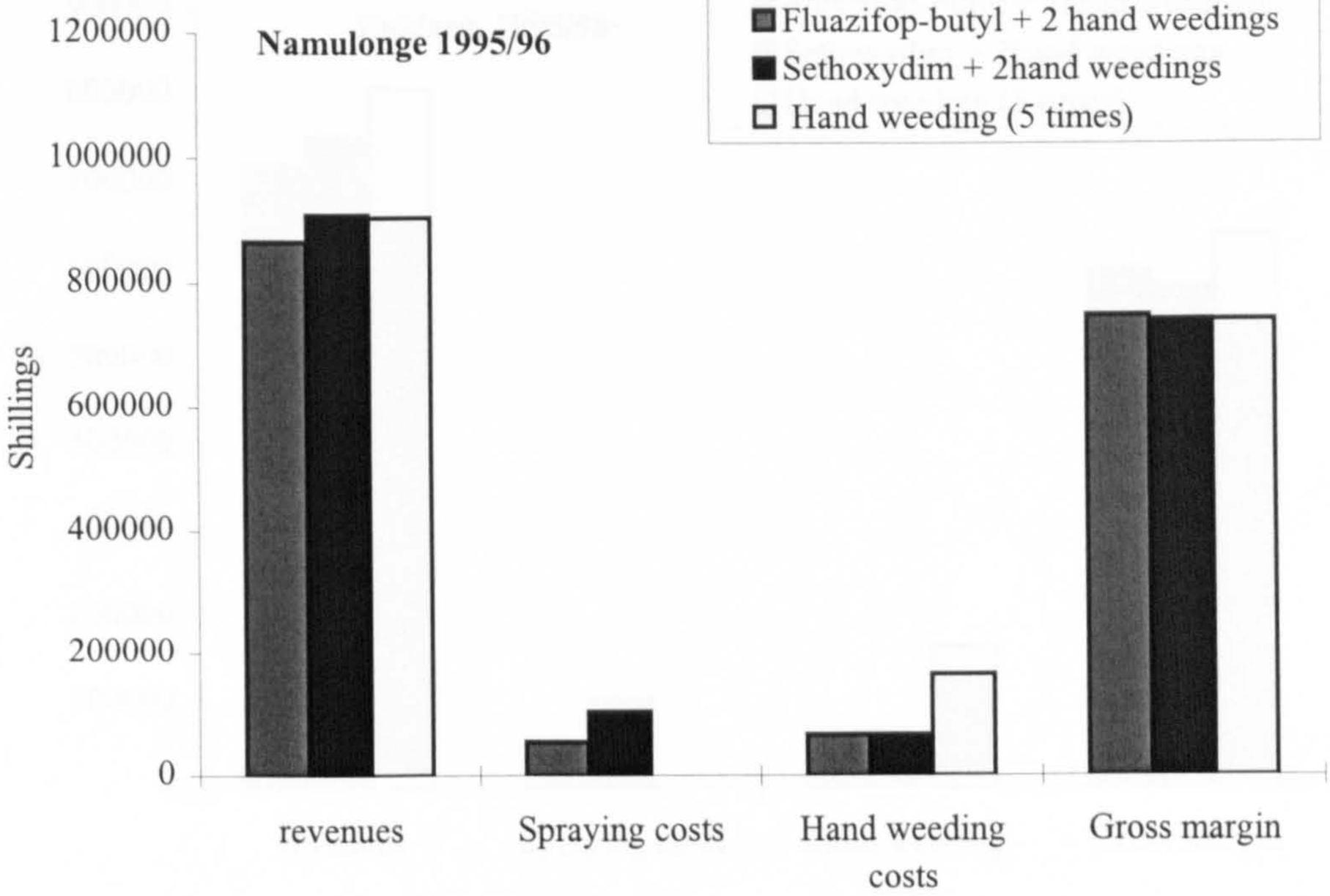


Figure 2.6 Effects of the weed control treatements on the economics of cotton production per hectare at Bukalasa.

calculated from the results achieved under each of the three application rates of each herbicide type. This analysis showed that the costs did not significantly differ amongst the treatments during the good season (1995/96) at the two sites. The net income however was noted highest from the combinations of fluazifop-butyl (162 g a.i.ha^{-1}) with 2 hand weedings from both locations. These results suggest opportunities to reduce the number of hand weedings and possibly save time and effort when compared to the current weeding practice (5 times). In the poor season (1997/98) the costs were significantly different amongst treatments, which seemed to have affected the net returns of some treatments. It was however evident that relatively high net returns were obtained from fluazifop-butyl (162 g a.i.ha^{-1}) combined with 2 hand weedings, which seemed to give convincing advantage over current weeding practice under Bukalasa conditions. The economic analysis based on averages (Figures 2.7 and 2.8) showed that the advantages of the spraying regimes over the current hand weeding practice (5 times) were not consistent between seasons (1995/96, a good season and 1997/98 a poor season), nor between the sites due to differences within the weather conditions of the two seasons. It was however, noted that the revenues and gross margins were highest at Bukalasa from fluazifop-butyl combinations during the poor season. It is however important to note the practical cash flow considerations for smallholder farmers with limited working reserves that may limit the extent to which they are able to take the advantage of spraying practice. However, given that most of the labour used at farm level is mainly family labour, the spraying regime might be practical for these smallholder farmers if they are able to divert the saved labour to profitably improve other income-earning crops such as coffee, maize, vegetables.



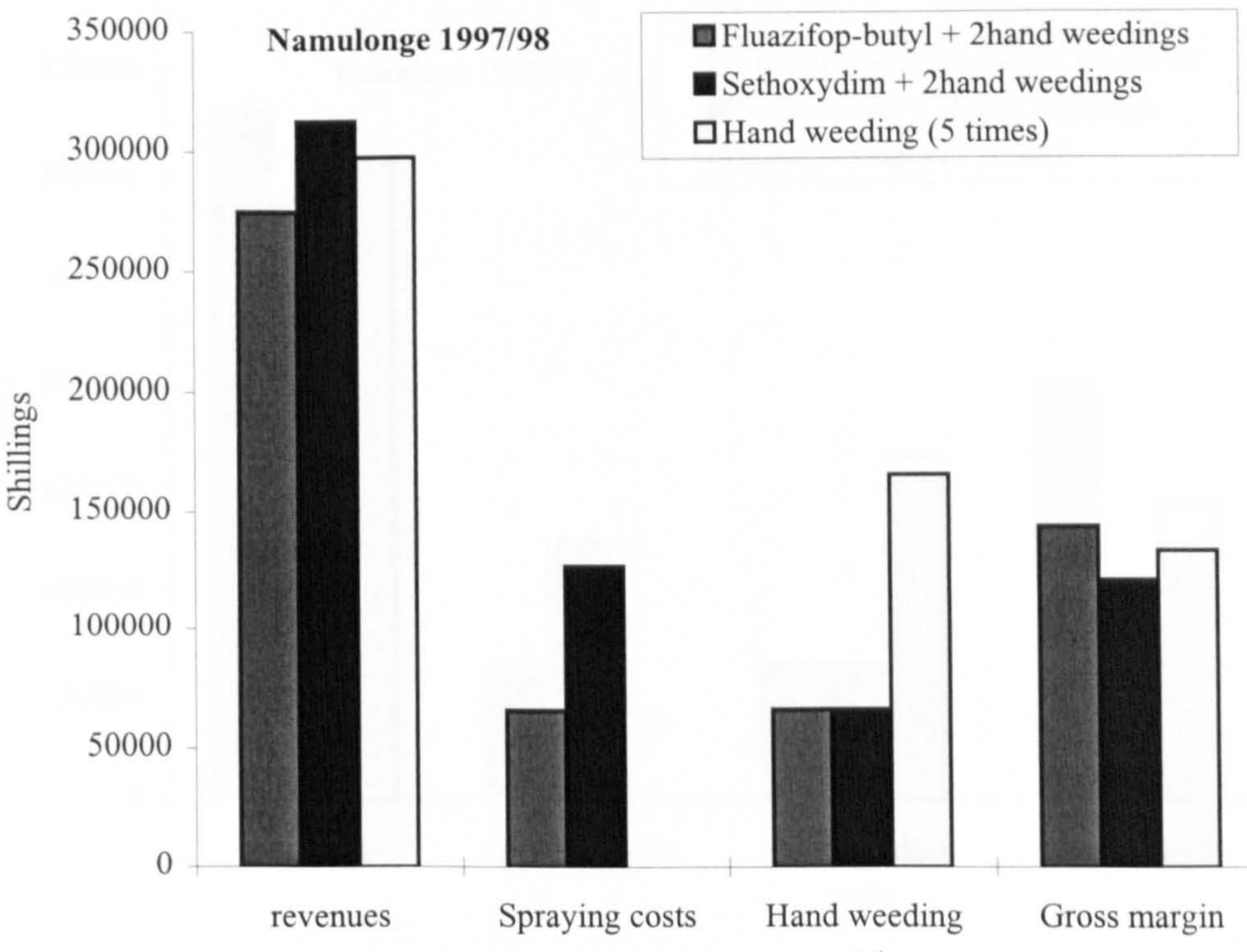
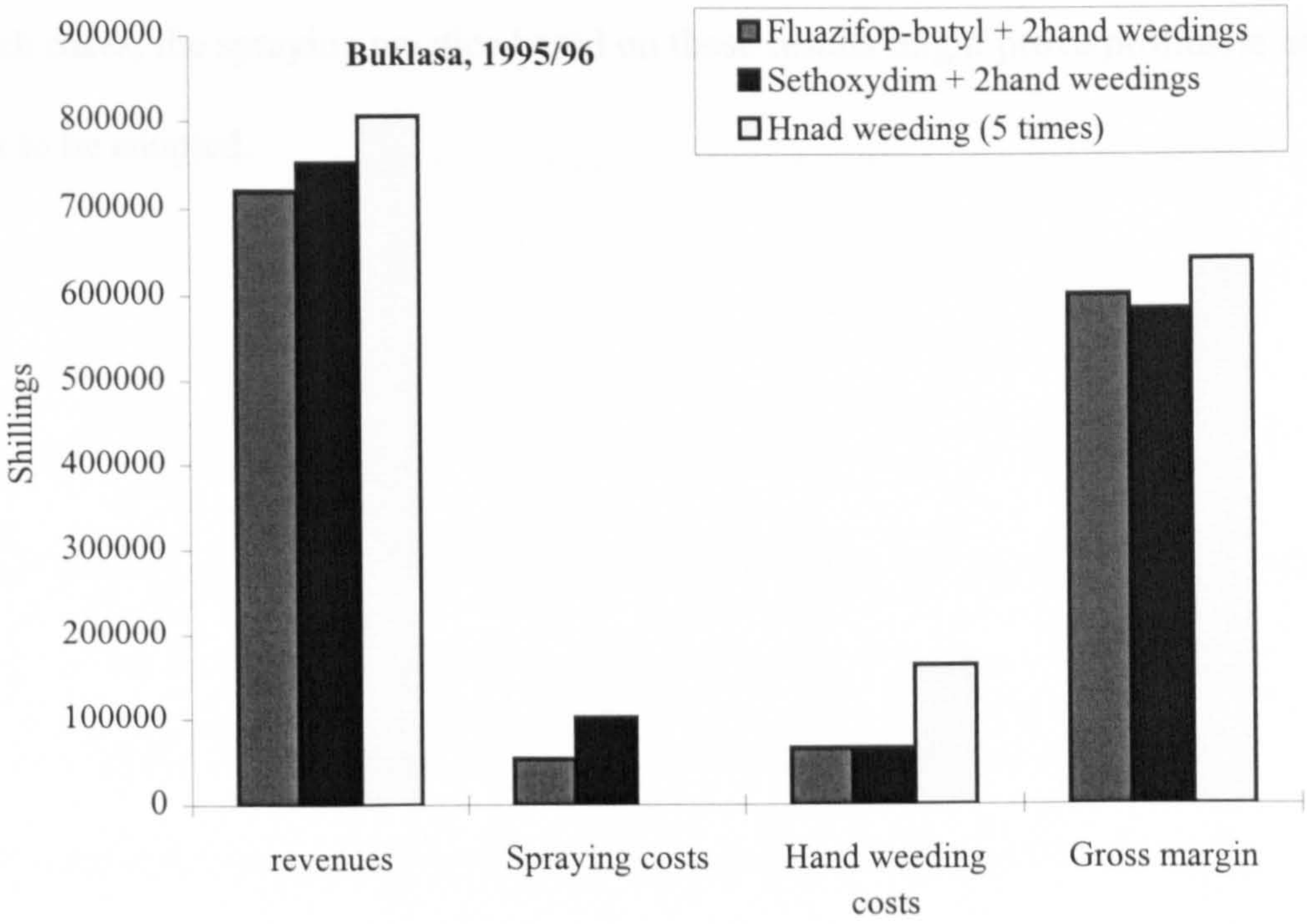


Figure 2.7 Average costs and benefits of the weed control methods used at Namulonge.



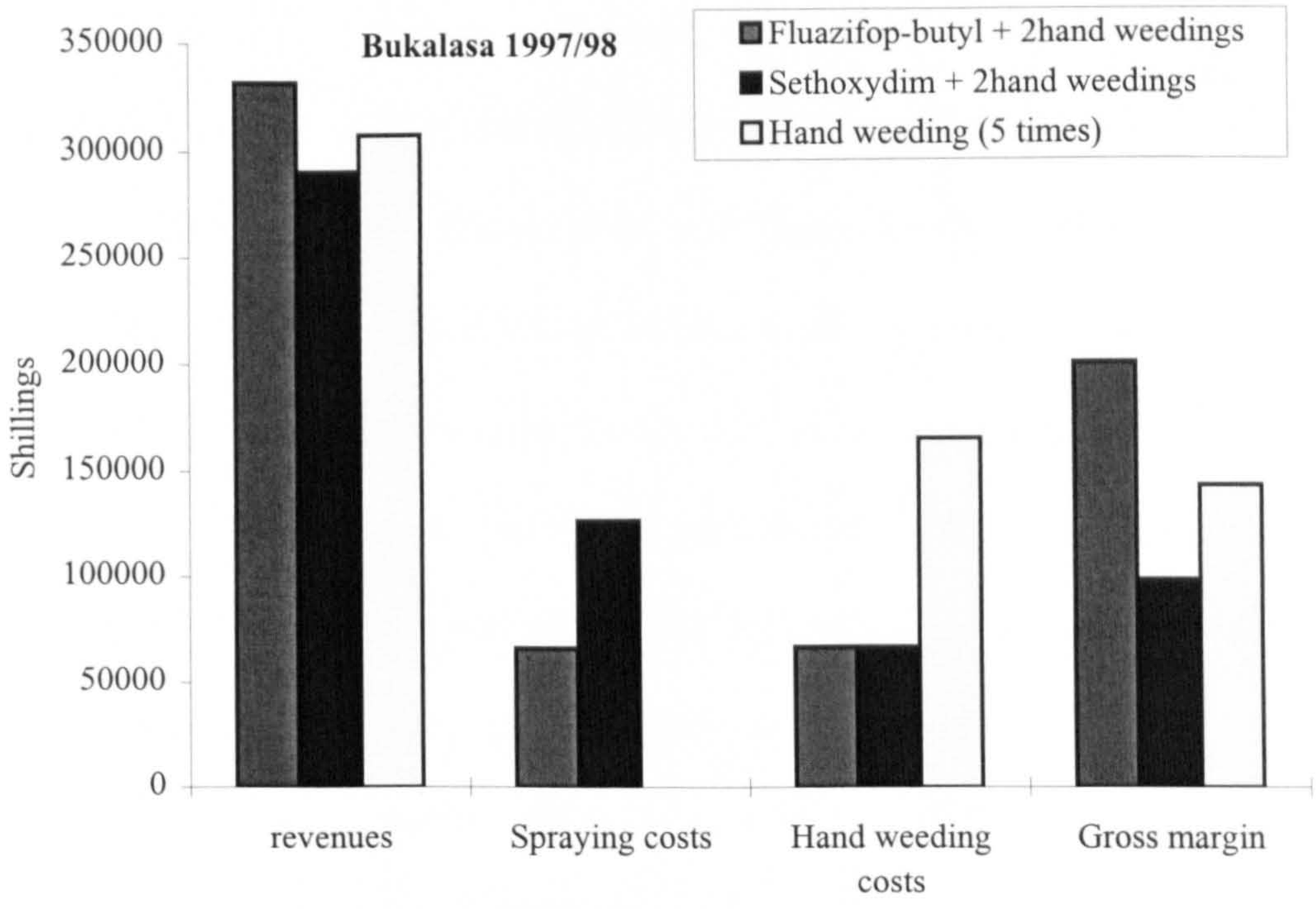


Figure 2.8. Average costs and benefits of the weed control methods used at Bukalasa.

In such cases, the spraying practice based on these results might prove profitable and thus likely to be adopted.

CHAPTER THREE

The activity of low concentrations of fluazifop-butyl and sethoxydim against *Digitaria abyssinica* under greenhouse conditions.

3.1 Introduction

Fluazifop-butyl (Fusilade) and sethoxydim (Checkmate) are post emergence herbicides active against both annual and perennial grass weeds (Hartzler *et al.*, 1983; Hays and Williams, 1981; Chandrasena and Sagar, 1987). These herbicides are foliage applied and highly systemic. Sethoxydim and fluazifop-butyl are translocated in the plant by xylem and phloem (Plowman *et al.*, 1980). Both herbicides have been extensively researched on but their mode of action is not yet understood. However, when applied to the plant they have been reported to cause general chlorosis in the susceptible grass weeds within 5 or more days after application (Chandrasena and Sagar, 1984). They observed that the chlorosis starts with the youngest leaves. Similar visual symptoms were observed on *D. abyssinica* under field conditions after the application of fluazifop-butyl and sethoxydim. And this led to initiation of the greenhouse experiment to effectively assess their injury activity against the weed. This was partly done by measuring fluorescence parameters of *D. abyssinica* leaves after treatment. Fluorescence parameters (Fo, Fv, Fm and Fv/Fm) have been widely used to evaluate the activity of foliar applied herbicides against weeds (Voss *et al*, 1984; Richard *et al.*, 1983). Fluorescence measurements can also be used to quantify the penetration of herbicides into the plant leaf where inhibition of photosynthetic electron transport by a herbicide results in a modification of the kinetics of chlorophyll

fluorescence due to increased dissipation of absorbed heat via chlorophyll fluorescence (Izawa and Good, 1965; Habash *et al.*, 1985). In other studies fluorescence has been reported as an indicator of weed resistance to herbicides (van Oorschot and Straathof, 1988).

objectives of the study are;

1) to investigate the response of *Digitaria abyssinica* to the low and high dose rates of fluazifop-utyl and sethoxydim.

2) to assess the injury caused by fluazifop-butyl and sethoxydim on *D. abyssinica* through measuring fluorescence parameters.

3.2 Methods and Materials

The experiment was conducted in the greenhouse at the University of Newcastle upon Tyne experimental station at Close House, Heddon on the Wall, Northumberland, UK in April 1997. The perennial weed *D. abyssinica* was investigated in the study and it was obtained from the cotton fields in Uganda (East Africa). *D. abyssinica* was propagated using rhizome fragments which consisted of 2-4 viable nodes. The fragments were placed in pots of 13 cm diameter at 3.2 cm depth in light sandy loam soil mixed with peat. The pots were placed in the glasshouse where temperatures were maintained at 20-30°C and relative humidity of 30-50%. Watering of the plants was always done twice a day. New plants from the viable rhizomes started sprouting 5-7 days after propagation. Three weeks after emergence, there was already an average of 8-10 actively growing shoots consisting of approximately the 4^{th} or 5^{th} node growth stage and with an average of 6-8 leaves per plant. It was at this stage that the weed was sprayed.

Herbicides used were fluazifop-butyl (Fusilade) emulsifiable concentrates (125g/L, EC) and sethoxydim (Checkmate) emulsifiable concentrates (193g/L, EC) plus the control where no herbicides were applied. The detailed treatments are described in the table below;

Table 3.1. Reduced herbicide dose rates applied on *D. abyssinica* in the greenhouse.

Treatment	Dose rates (g a.i.ha^{-1}	Proportion of full rate (%)
Fluazifop-butyl (fusilade)	138	70
Fluazifop-butyl (fusilade)	162	85
Fluazifop-butyl (fusilade)	188	100
Sethoxydim (chekmate)	405	70
Sethoxydim (checkmate)	502	85
Sethoxydim (checkmate)	579	100
Control	0	0

The above dose rates were obtained by reducing the full rates of each herbicide by 15 and 30%.

The experiment was replicated six times in a complete randomised design (CRD) as illustrated below;

Treatment	1	2	3	4	5	6
A	1D	2A	3G	4C	5E	6H
B	1E	2H	3B	4F	5D	6A
C	1A	2C	3D	4E	5H	6F
D	1G	2F	3A	4B	5C	6G
E	1B	2E	3H	4D	5F	6E
F	1H	2D	3C	4G	5A	6B
G	1C	2B	3F	4H	5B	6C
H	1F	2G	3E	4A	5G	6D

1-6 represents the number of replications, A-H represents the number of treatments

Herbicides were applied on actively growing plants of *D. abyssinica* using a moving track sprayer equipped with Teejet flat fan nozzles 8002 (Spraying Systems Co., Wheaton) at 2 kPa. The sprayed plants were returned to the greenhouse a few minutes after treatment. The herbicides were assessed using visual observation (chlorosis and necrosis), fresh and dry weights of shoots and rhizomes, and leaves fluorescence in *D. abyssinica*.

3.2.1 Measurement of fresh and dry weight of shoots and rhizomes

The weights of fresh shoots and rhizomes of *D. abyssinica* were determined at the end of the experiment, 28 days after herbicide application. The fresh shoots and rhizomes samples were placed in an oven at 90°C for 48 hours. Thereafter they were weighed to determine their dry weights. Both fresh and dry weights of *D. abyssinica* shoots and rhizomes were determined by using a Electronic Precision balance, Precisa, 80A/300M/159M, 300C (PAG Oerlikon AG, Zurich-Switzerland).

3.2.2 Fluorescence measurements

Fluorescence was measured fluorescence was measured at hour 4 after the application of the herbicides. Subsequent measurements were taken at 1, 2, 3, 4, 5, 14, 21, and 28 days after herbicide application. The fluorescence parameters are defined as: Fo- initial rise of fluorescence when dark adapted leaf is illuminated with a flash of light; Fm- maximum fluorescence value obtained from a particular amount of light intensity; Fv- variable component in fluorescence of Fo and Fm; Fv/Fm- the ratio of variable fluorescence and maximal fluorescence. Fluorescence measurements of *D. abyssinica* leaves after treatment were done both in the field and greenhouse. In both conditions these parameters were

measured using a Plant Efficiency Analyser (PEA) Hansatech Ltd, Norwich, UK. The PEA consists of ;

- Leaf clip used for dark adaption of the fresh green leaf.

-Sensor plug unit provides illumination of the leaf and detects consequent fluorescence signal.

- Control box is used for digitising fluorescence signal received by the sensor unit.

During the fluorescence measurements the leaf clip was attached to fresh leaf (one of the top three expanded leaves) on the plant and a small shutter plate was used to cover the leaf so that light was excluded and the dark-adaption takes place. During this period of dark-adaption fluorescence yield is quenched (Friesner and Won 1989; Krause and Weis, 1991). This process takes a variable amount of time depending on the plant species, light level prior to the dark transition and the condition of the plant (stressed or not). Calibration of time required for effective dark-adaption for *D. abyssinica* leaves was done. This was done by dark-adapting a number of leaves. The readings were taken at different times, 15, 20 and 30 minutes after dark adaption. No significant difference was noted between the times, hence 15 minutes was commonly used for fluorescence measurements in leaves of *D. abyssinica*.

3.2.3. Data analysis

Data collected on the fluorescence parameters and fresh and dry weights were subjected to analysis of variance (ANOVA). The means were separated at 5% level significance using Tukey's multiple range test.

3.3 Results and Discussion

3.3.1 Effect of fluazifop-butyl and sethoxydim on the fresh and dry weights of *D.abyssinica* shoots and rhizomes.

D. abyssinica shoots are described as the above ground portions of the plants and the below ground parts are rhizomes and fibrous roots. Prior to shoot and rhizome biomass assessment, visual symptoms on *D. abyssinica* showed that there was chlorosis and necrosis of the leaves, 7 and 21 days after herbicide application (Figures 3.1 and 3.2). These symptoms gave the first indication of *D. abyssinica* susceptibility to fluazifop-butyl and sethoxydim. Similar visual symptoms on other grass weed species due to sethoxydim and fluazifop-butyl have been reported by Magallanes *et al.*, (1986) and Asare-Boamah and Fletcher, (1983). In the present study data has showed a significant reduction of the fresh and dry shoots of *D.abyssinica* following the application of sethoxydim and fluazifop-butyl (Figure 3.3). These results suggested that the yellowing of *D. abyssinica* leaves (chlorosis) could have an early indication of plant retardation which led to the decrease of fresh and dry biomass. Stunting of crabgrass (*Digitaria* sp.) due to sethoxydim has been reported by Asare-Boamah and Fletcher, (1983). According to Swisher and Corbin, (1982); Anderson, (1983); Asare-Boamah and Fletcher, (1983) retardation of plants after the application of fluazifop-butyl and sethoxydim is associated with inhibition of cell division in meristems.

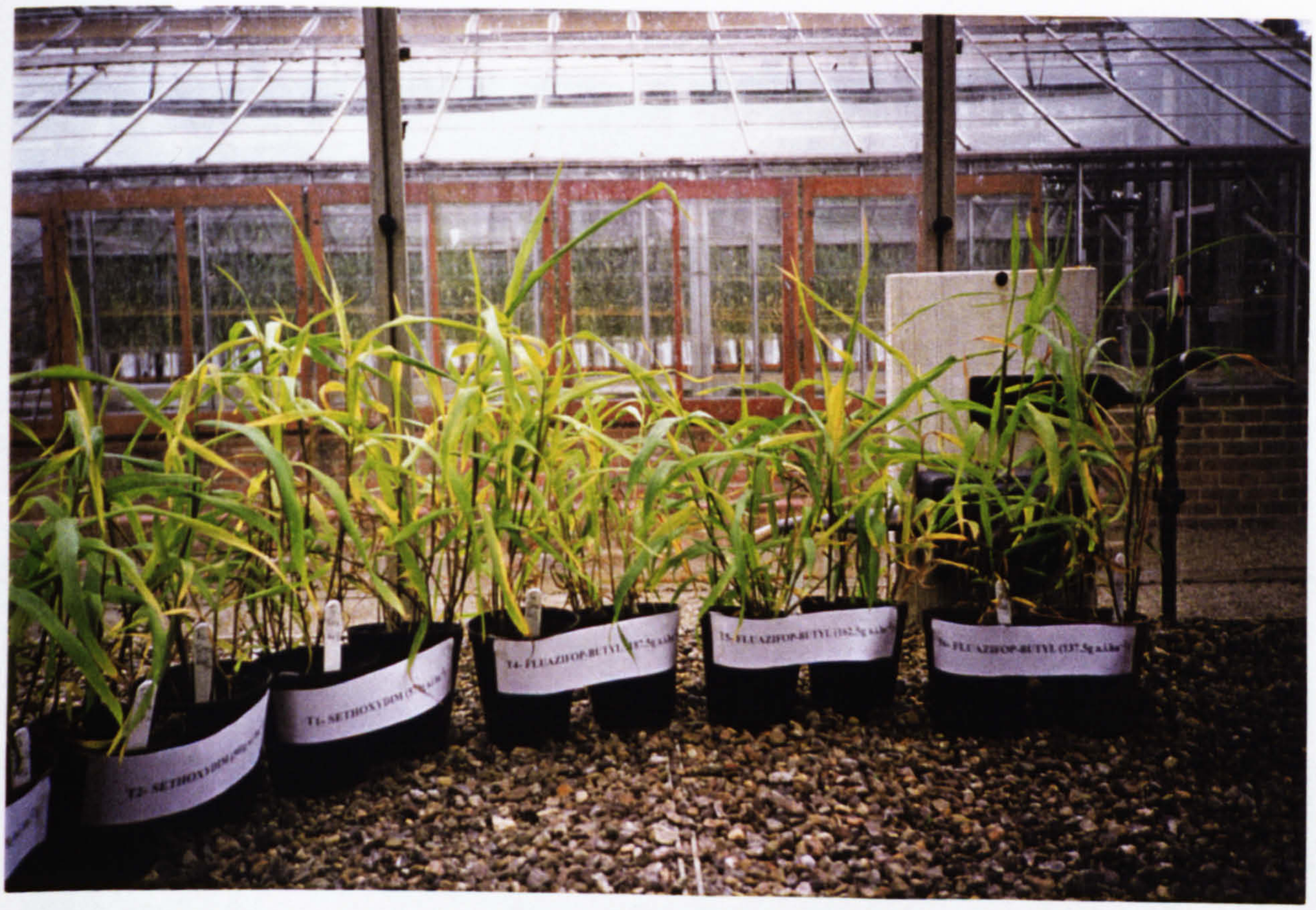


Figure 3.1. Chlorosis of *D. abyssinica* leaves observed 7 days after the application of fluazifop-butyl and sethoxydim (left to right are sethoxydim and fluazifop-butyl dose rates repsctively).

CHAPTER THREE

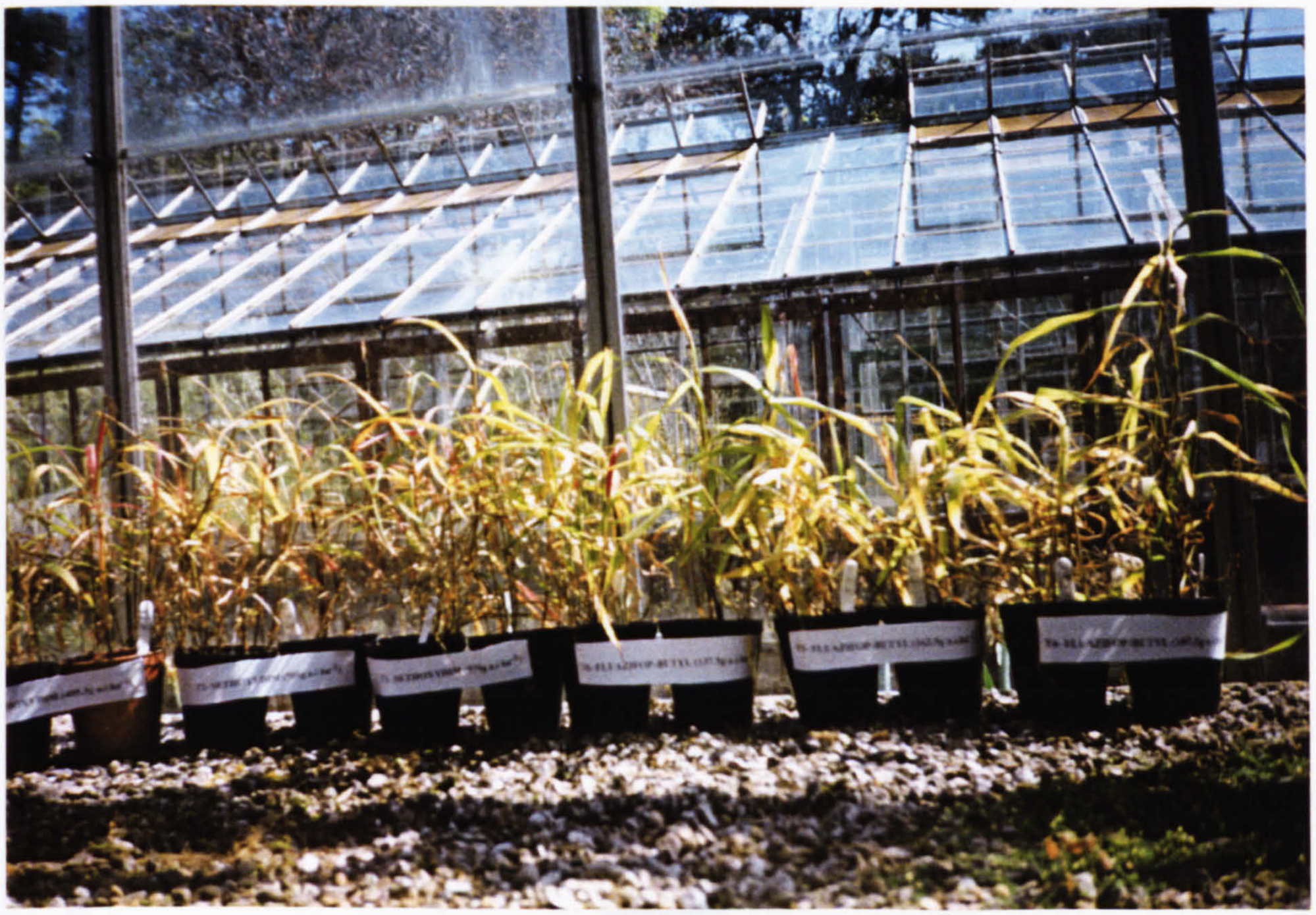
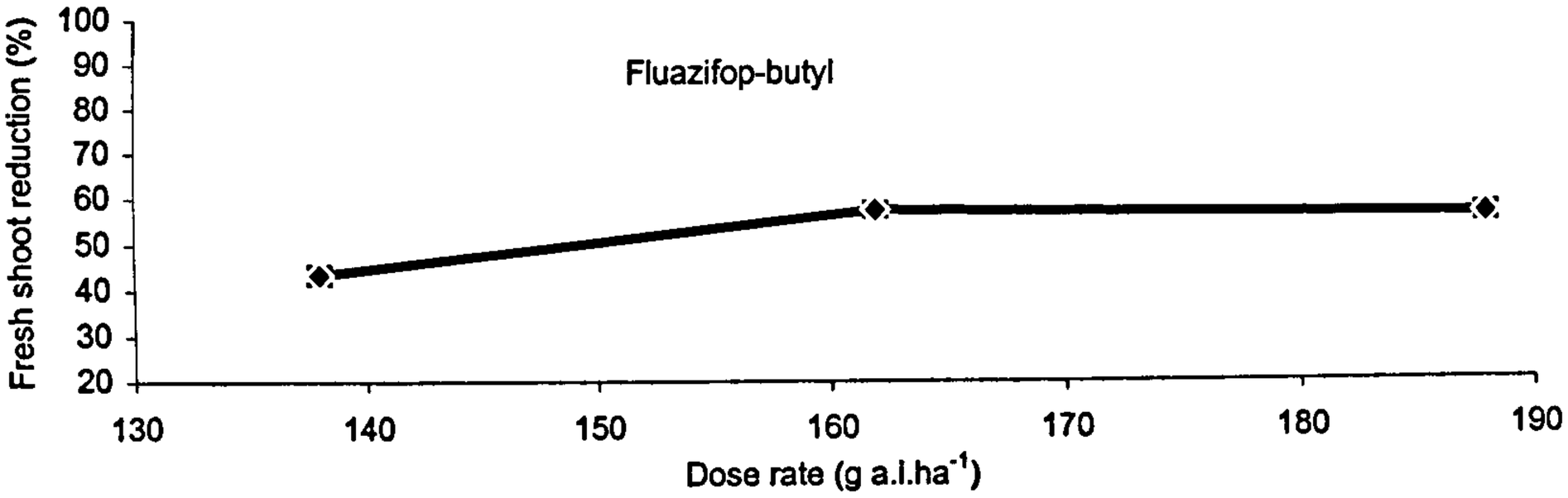
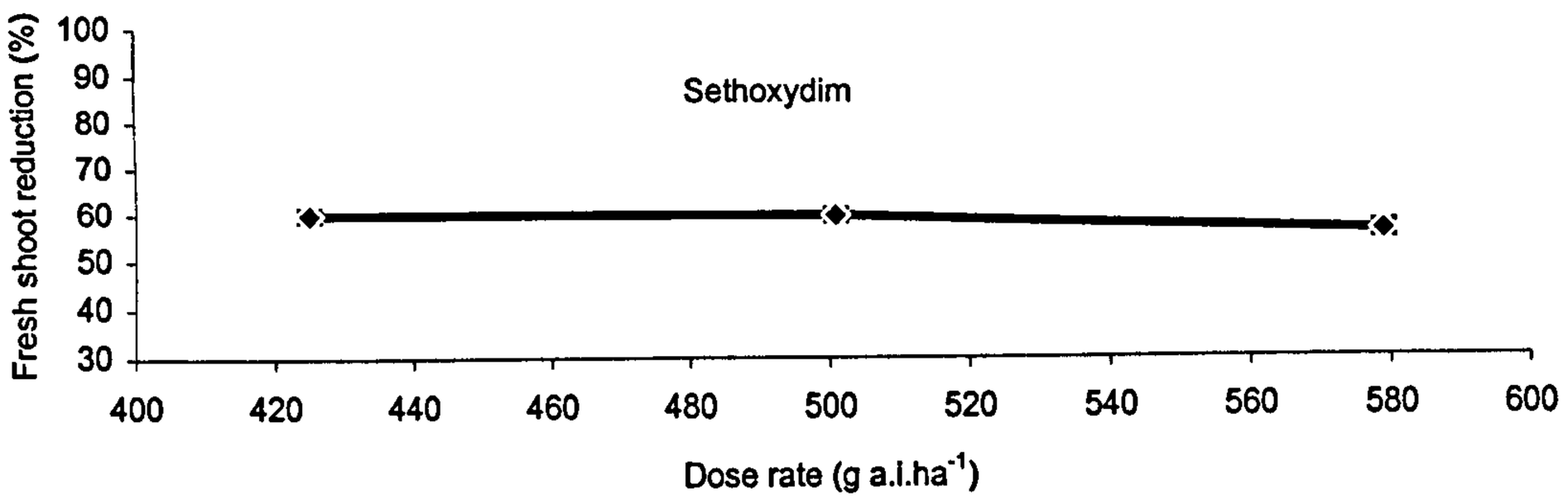
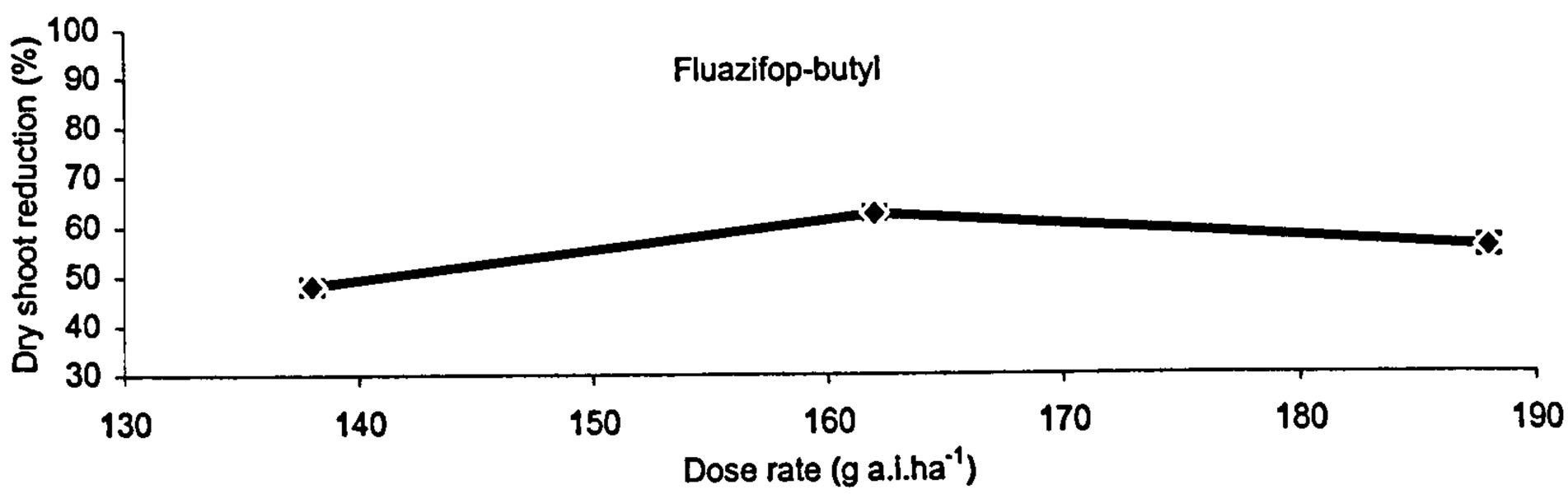


Figure 3.2. Necrosis of *D. abyssinica* leaves observed 21 days after the application of fluazifop-butyl and sethoxydim (left to right are sethoxydim and fluazifop-butyl dose rates respectively).







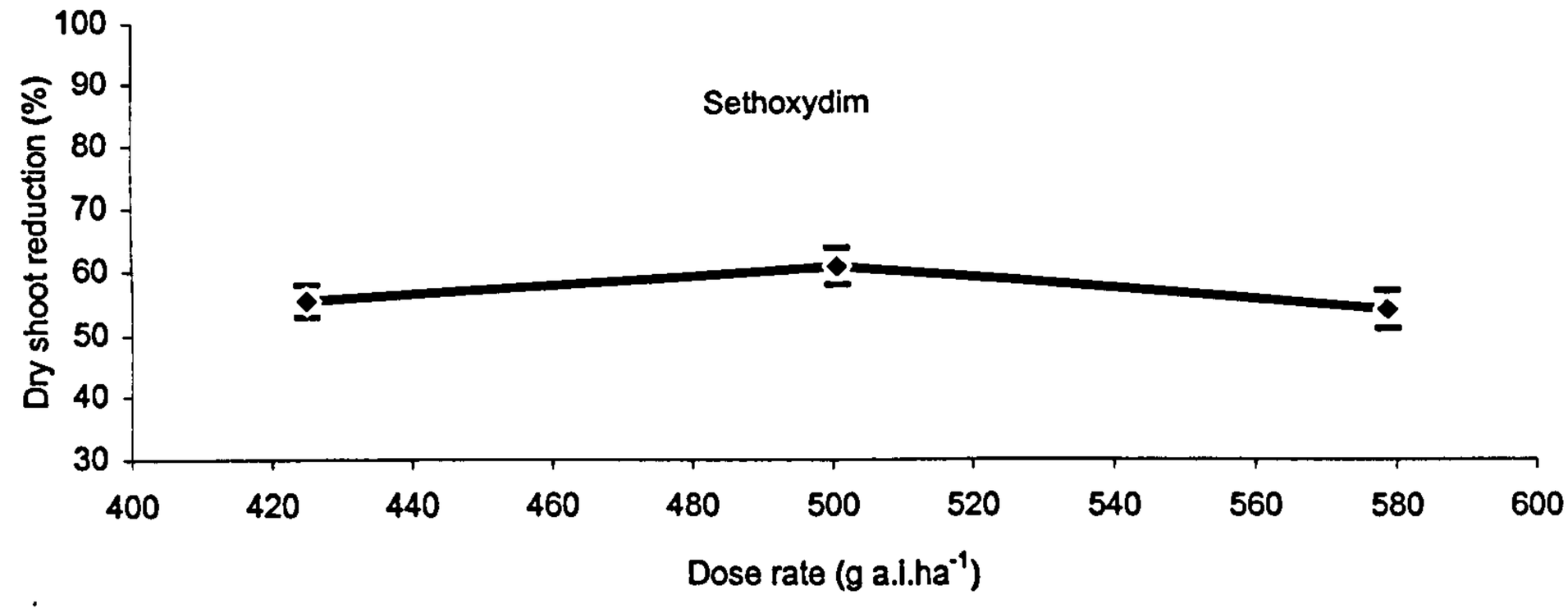


Figure 3.3. Percentage reduction of fresh and dry shoots of *D. abyssinica* after treatment with the herbicides in the greenhouse. Bars represent standard error of each mean value of six replications.

Following the results obtained in this study, analysis of variance indicated that there was no significant difference in the percentage reduction of the fresh and dry shoots among the dose rates of both herbicides but it was noted between treated and untreated plants(Appendices 3.1 and 3.2). Thus the percentage reduction of the dry weight corresponded with the one of fresh weight. Results indicated high activities of fluazifop-butyl and sethoxydim against *D. abyssinica* and the phytotoxic action of the reduced dose rates in comparison to the full dose rates. Swisher and Corbin, (1982) and Carr *et al.*, (1986) have associated the activities of sethoxydim and fluazifop-butyl against susceptible grass weed species with their uptake and penetration into the plant. Figure 3.4 illustrates the reduction of the fresh and dry weights of *Digitaria* rhizomes as a percentage control of the treated plants. It was found that although sethoxydim and fluazifop-butyl are foliage applied herbicides, they affected the rhizomes. The herbicides significantly decreased the fresh and dry rhizomes of *D. abyssinica* (Appendices 3.3 and 3.4). It was noted that the percentage reduction was even higher in the rhizomes than the shoots. These results supported other research findings on the control of rhizomes of other perennial grass weeds with sethoxydim and fluazifop-butyl (Sarpe and Dinu, 1980). On the hand the effective control of *Digitaria* rhizomes with these herbicides could have been due to their high systemicity as they are translocated throughout the plants thus not only the aerial portions of the plants were killed. Translocation of sethoxydim and fluazifop-butyl in the plants has been widely studied (Plowman *et al.*, 1980; Chandrasena and Sagar, 1984; Kells *et al.*, 1984). The present study has showed that the dose rates applied on *D. abyssinica* equally affected the shoots and the rhizomes.

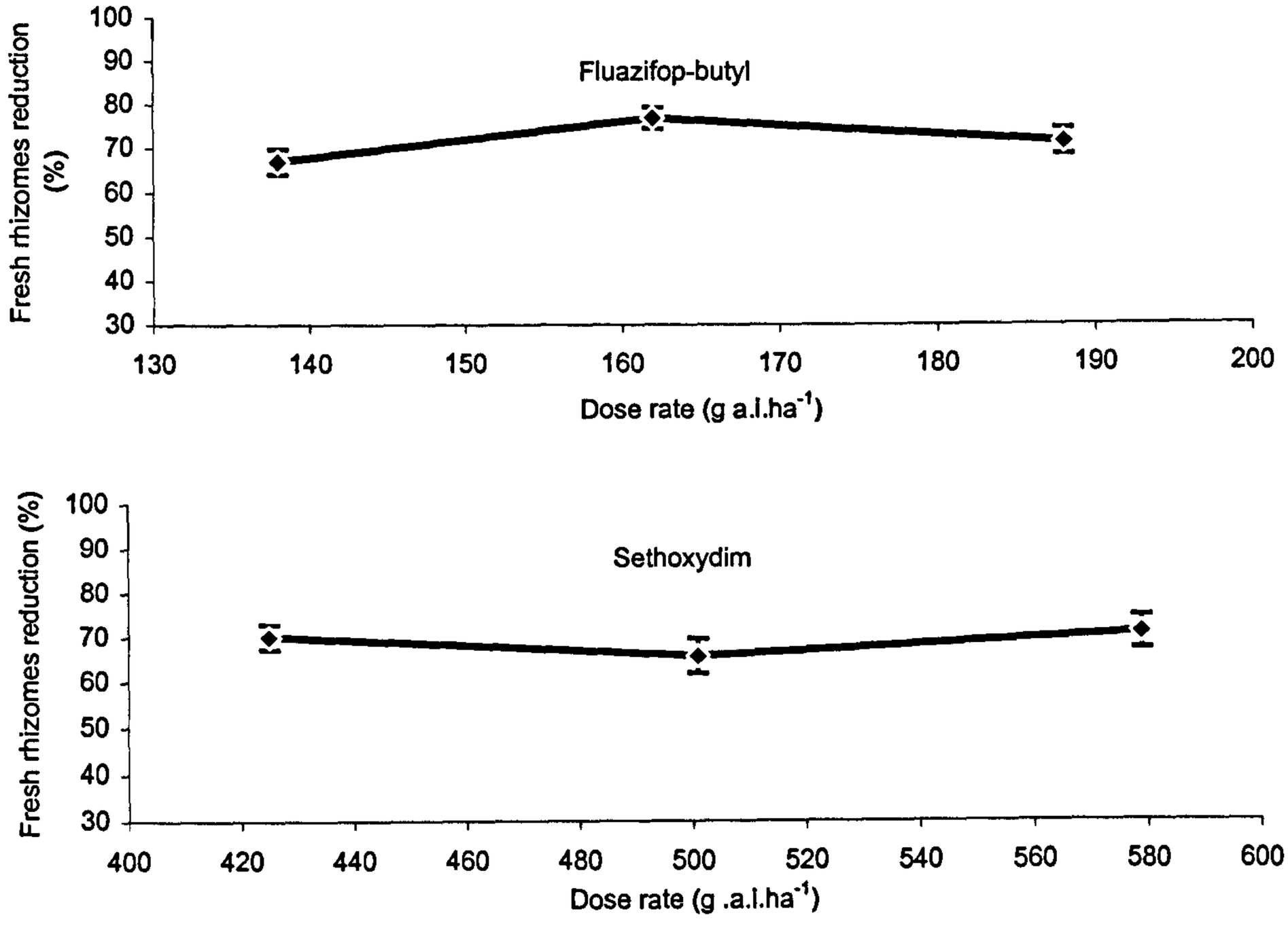
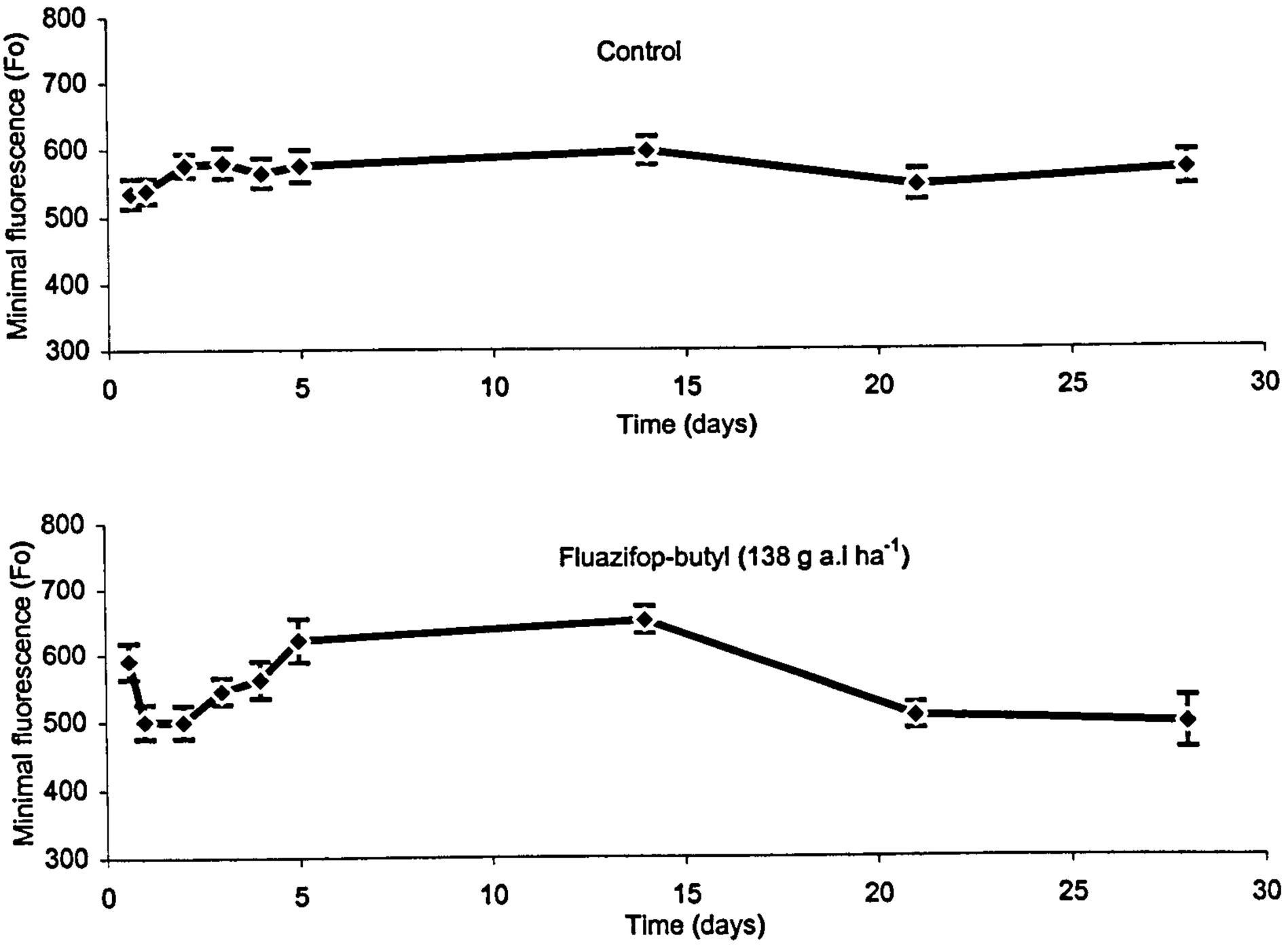


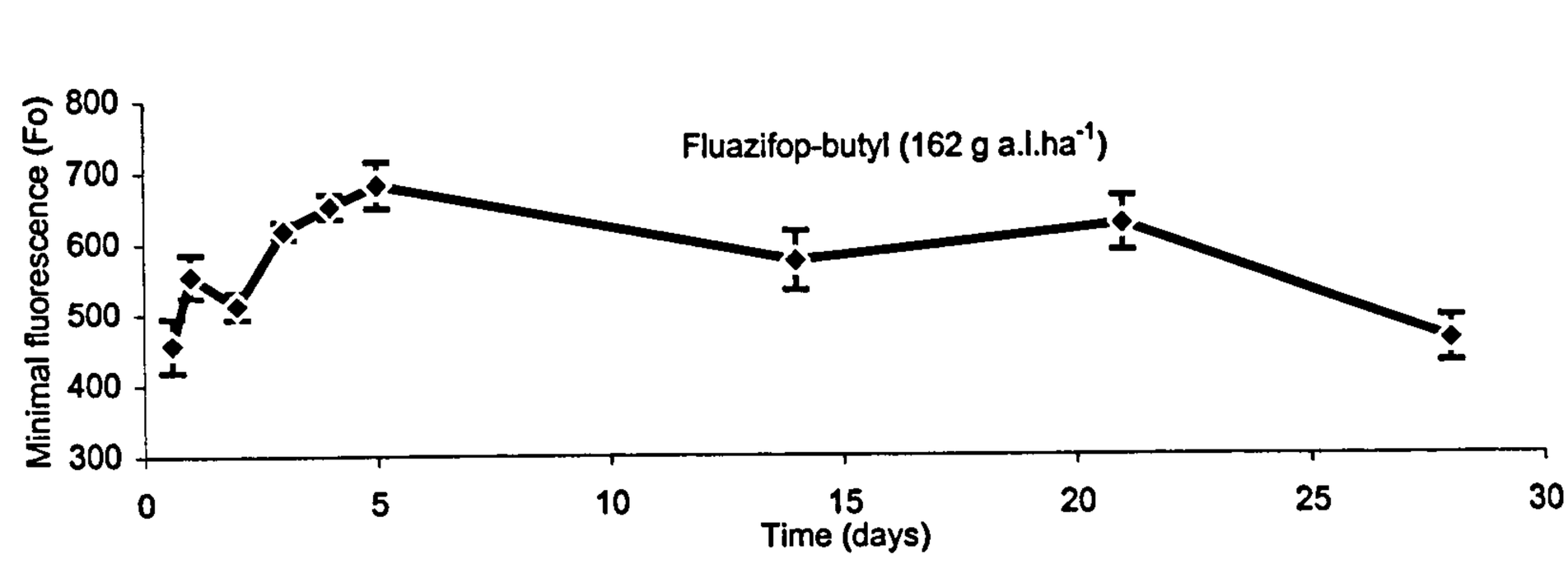
Figure 3.4. Percentage reduction of fresh and dry weights of *D. abyssinica* frhizomes obtained after the application of herbicides in the greenhouse.
Bars represent standard error of each mean value of six replications.

3.3.2 Fluorescence of *D. abyssinica* leaves after treatment with fluazifop-butyl and sethoxydim

Light energy absorbed by the chlorophyll molecules in a leaf is emitted as fluorescence. This light can be used to drive photosynthesis (photochemistry), excess energy is dissipated as heat or it can be re-emitted as light (chlorophyll fluorescence) (Maxwell and Johnson, 2000). Whilst the amount of chlorophyll fluorescence is a relatively small percentage of the total light absorbed (1 or 2%). It is important to note that the measurements of fluorescence can only be relative since light is usually inevitably lost. Thus the analysis must include some form of normalisation with a wide variety of different fluorescence parameters being calculated. During dark adaptation in leaves there is no chance for photochemistry to occur. The initial steep rise in fluorescence, Fo, which happens within seconds after a flash of light, originates from the antenna chlorophylls in PSII before the energy is transferred to the reaction centre (Schreiber and Bilger, 1987). Thus Fo measures the minimum fluorescence signal when reaction centres are open, whereas Fm measures the maximum fluorescence signal once all the reaction centres have been closed by the saturating pulse of light. Fluorescence has been used as a fast and non destructive method to assess the activity of herbicides on photosynthesis (Schreiber *et al.*, 1977). Photosynthesis measurements such as fluorescence could be a suitable method to detect plant susceptibility or tolerance (Hubbard and Whitwell, 1991).

In the present study, the fluorescence curves obtained from *D. abyssinica* leaves treated with fluazifop-butyl are illustrated (Figures 3.5, 3.6 and 3.7). Results showed that fluorescence minimal (Fo) obtained at different times in the untreated leaves relatively constant compared to the treated leaves (Figure 3.5). Fo increased at certain times after application in the leaves treated with fluazifop-butyl unlike in the untreated leaves. The increase of the Fo in the treated leaves of *D. abyssinica* might have suggested damage of the leaves due to the herbicide. As a result there was disruption of the normal fluorescence of *D . abyssinica*. These results supported results reported by Chandrasena and Sagar, (1987) when they applied fluazifop-butyl on *Elymus repens* (L) Gould. Research elsewhere has also showed increase of fluorescence (Fo) in treated leaves of susceptible plants after the application of various herbicides (Gasquez *et al.*, 1982; Retzlaff *et al.*, 1979; Ahrens *et al.*, 1981). According to Habash *et al.*, (1985) increase of Fo is an indication that the herbicides might have affected the reaction centres in PSII, thus blocking the transfer of the electrons, and as a result the excitation energy builds up in PSII leading to an increase of Fo emission. The present results, however, showed that the increase of Fo occurred at different times under different dose rates, which could have suggested that the effectiveness of these dose rates against *D. abyssinica* varied with time. Results obtained on Fv/Fm are given (Figure 3.6). Fv/Fm is an important non-destructive measure of the photosynthetic apparatus (Björkman and Demmig, 1987), and it can be used as a good indicator for photochemical efficiency in the plant (Demmig and Björkman, 1987).





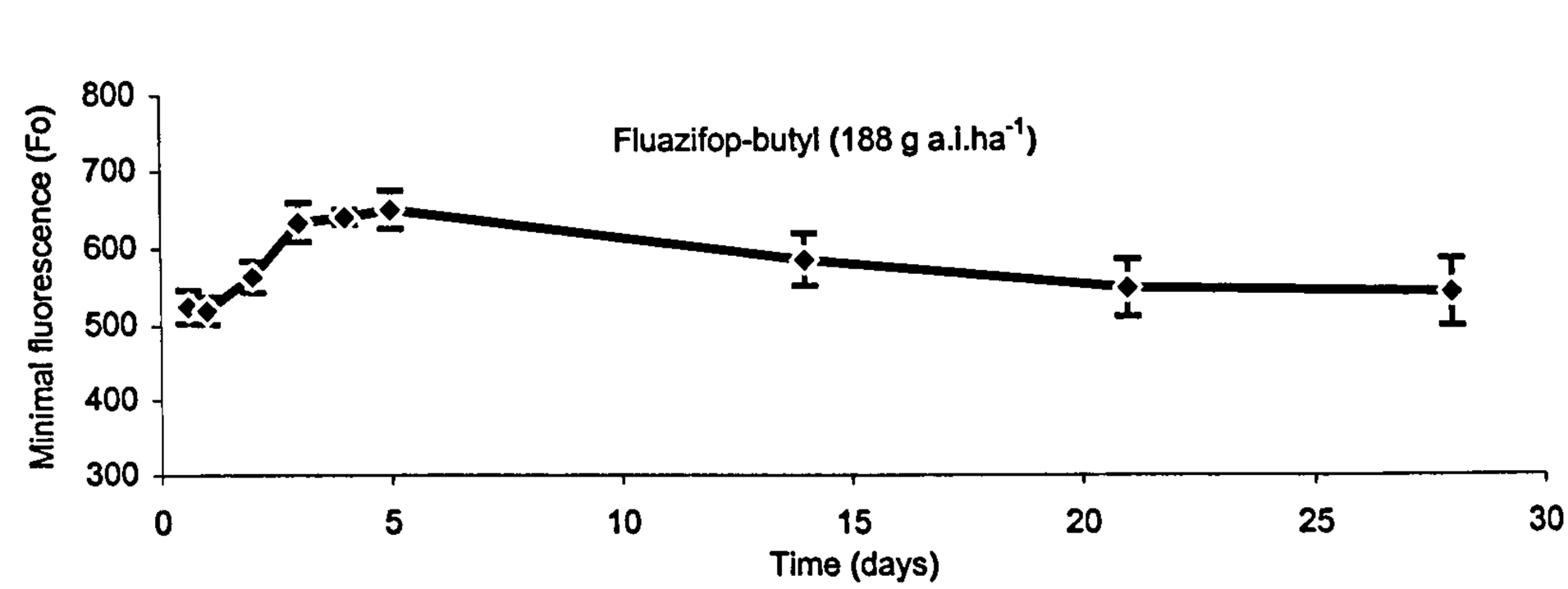


Figure 3.5. Minimal fluorescence (Fo) of *D. abyssinica* leaves measured at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of six replications.

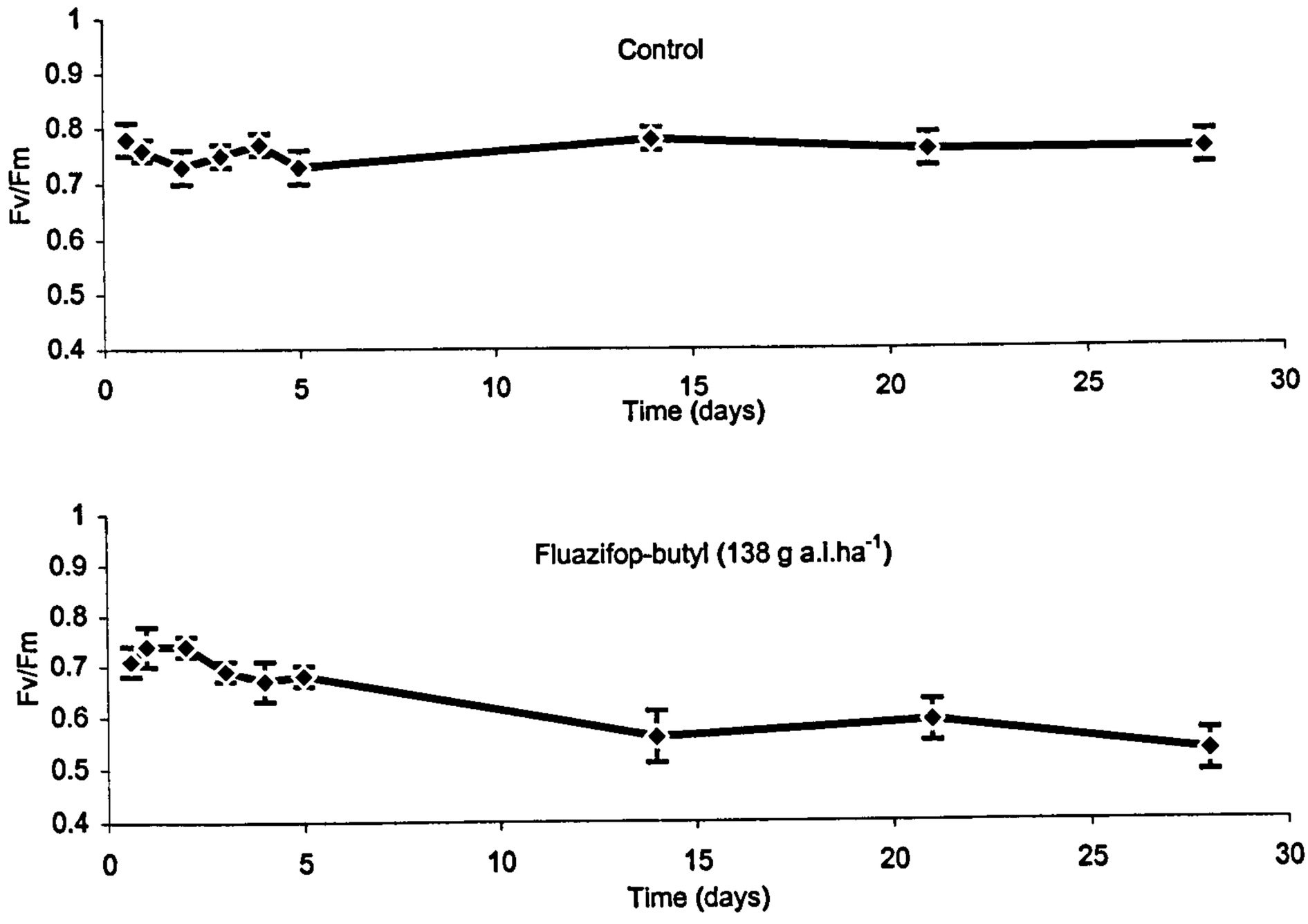
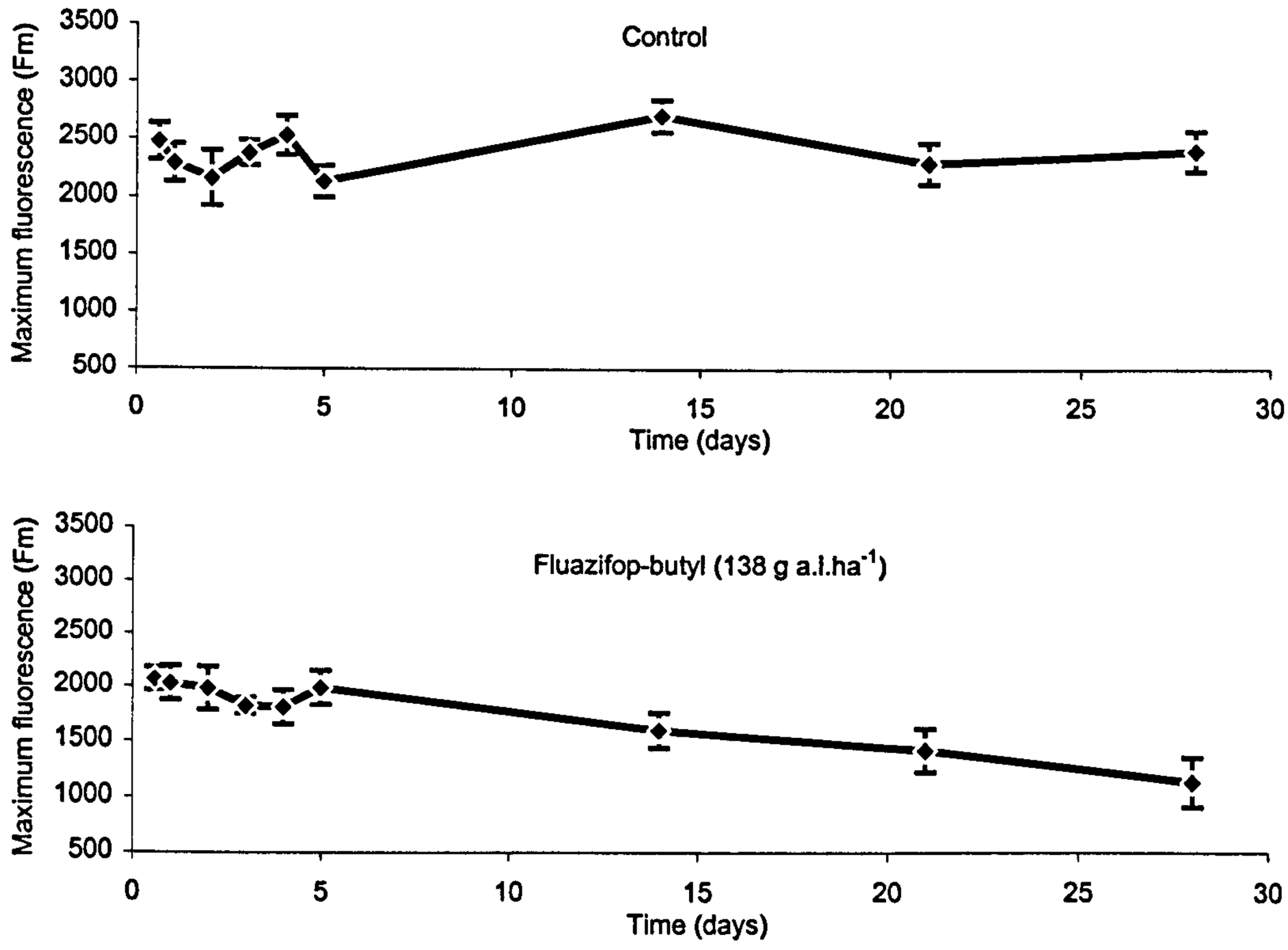
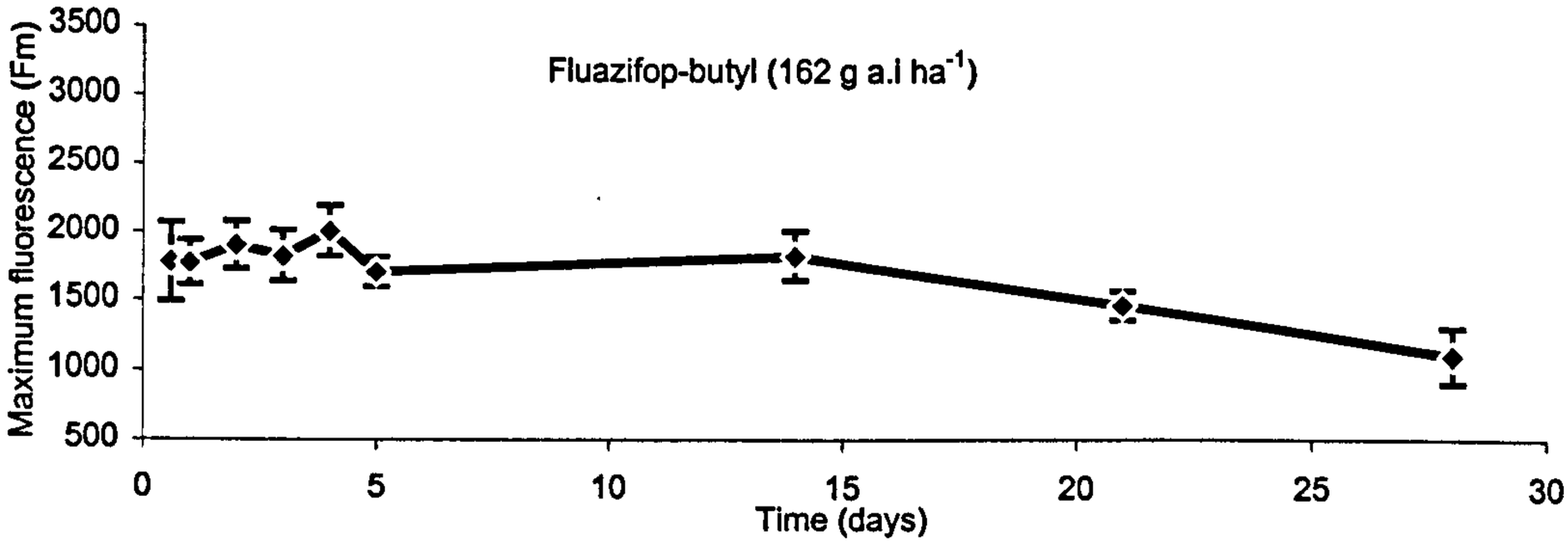


Figure 3.6. Fv/Fm of *D. abyssinica* leaves measured at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of six replications.

The present results indicated that the application of fluazifop-butyl significantly decrease in Fv/Fm of *D. abyssinica* leaves at times. It was revealed that the times at which Fo increased, Fv/Fm was decreased. For example fluazifop-butyl at 138 g a.i.ha^{-1} gave a Fo increase at day 14, this has been reflected in the decrease of Fv/Fm (0.56) compared to the ratio obtained from untreated leaves (0.78) at the same time. The Fv/Fm results gave an additional good logical indication of the herbicide toxicity on *D. abyssinica*. Reduction of Fv/Fm following the application of fluazifop-butyl on other grass weeds has been reported elsewhere (Chandrasena and Sagar, 1987). It was, however, noted in the study that at later times of 21 and 28 days Fv/Fm decreased irrespective of the low Fo obtained during these periods. This decrease could have been due to the fluorescence maximum (Fm) which gave relatively higher valves compared to the Fo, and possibly indicating stress of the *D. abyssinica* plants (Figure 3.7). As it was indicated by Kitajima and Butler, 1975; Bolhar-Nordenkampf and Öquist, 1993 that fluorescence can reach its maximum or peak when all reaction centres are closed. A similar situation might have occurred in the treated leaves of *D. abyssinica.* Sethoxydim seemed to have had a similar trend of fluorescence fluctuation at times after treatment compared to fluazifop-butyl. Fluorescence values obtained from *D. abyssinica* leaves treated with sethoxydim are given (Figure 3.8, 3.9 and 3.10). Results showed that all sethoxydim dose rates gave high minimal fluorescence (Fo) values in the treated leaves immediately after application at hour 4(Figure 3.8).





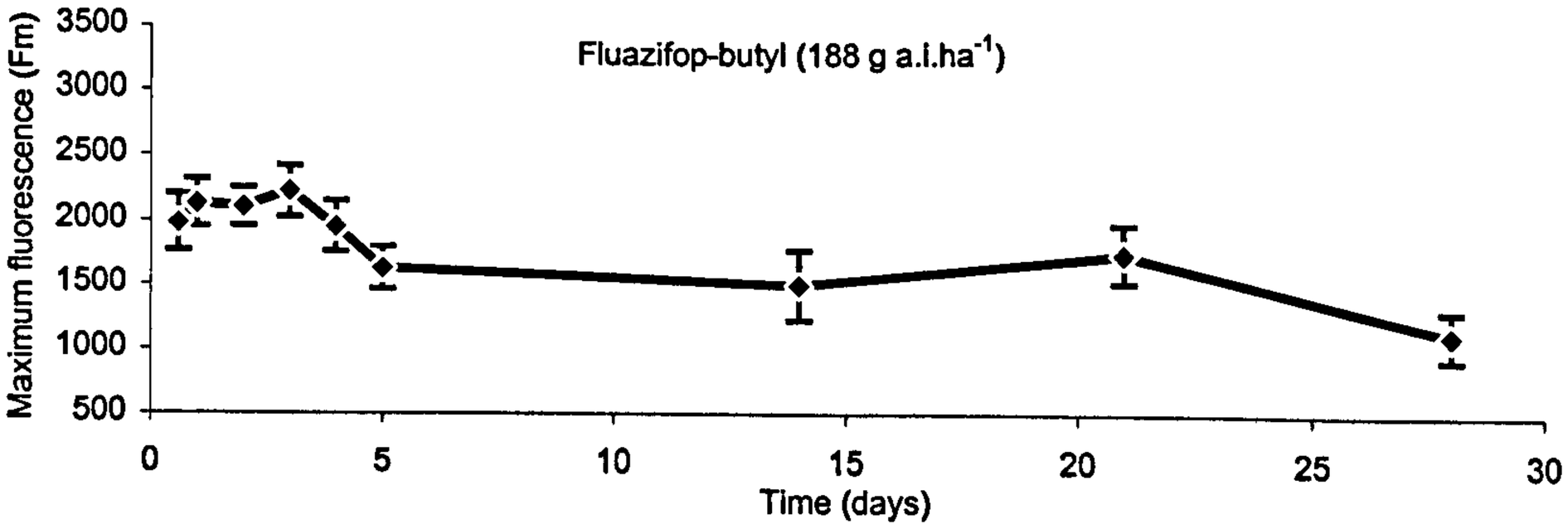
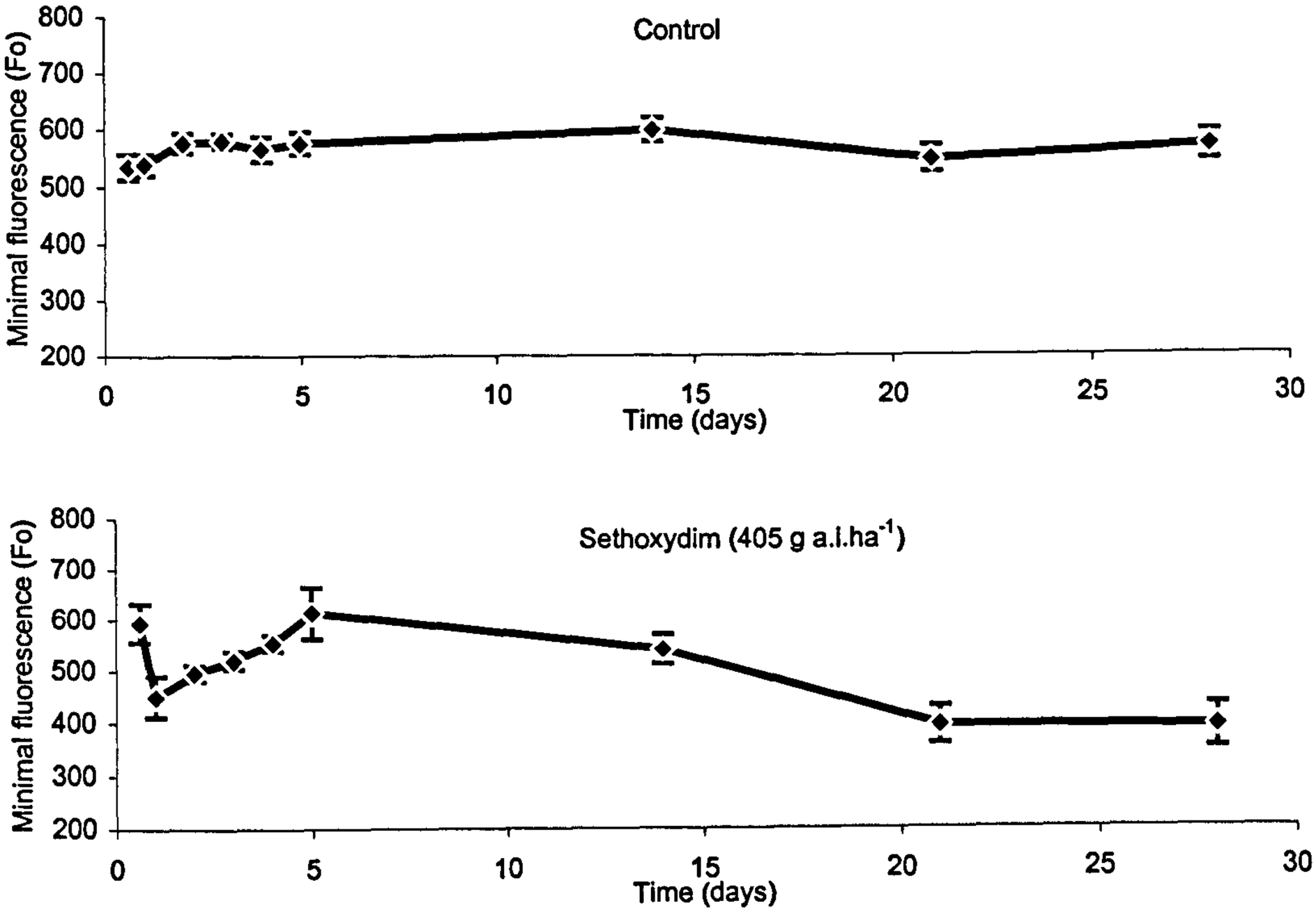
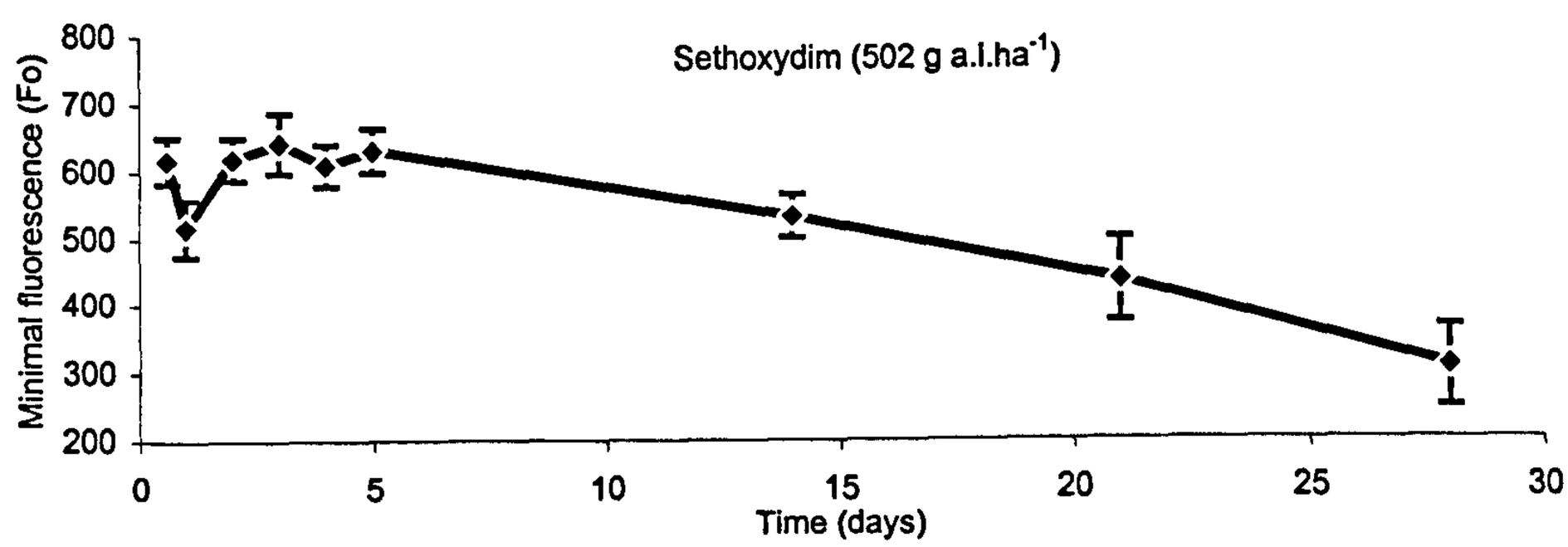


Figure 3.7. Maximum fluorescence (Fm) of *D. abyssinica* leaves obtained at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of six replications.





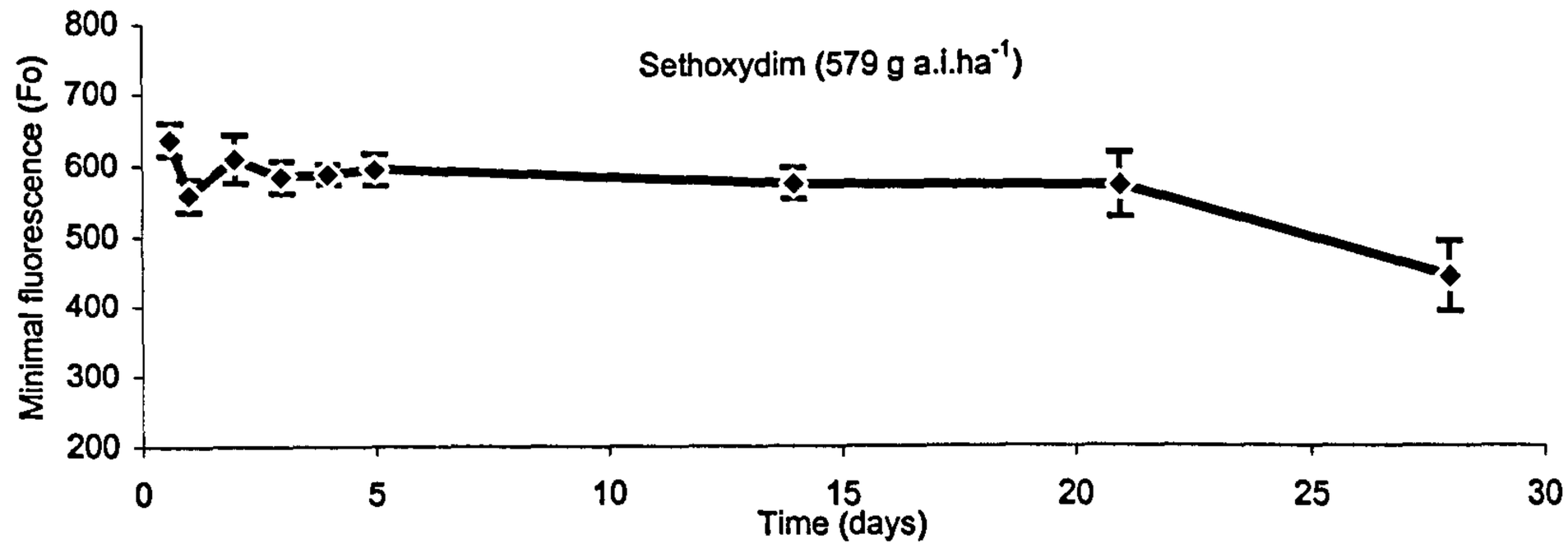


Figure 3.8. Minimum fluorescence (Fo) of *D. abyssinica* measured at times after different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of six replications.

The values ranged between 590 and 637 compared to the control (533). The increase of fluorescence (Fo) shortly after the application of sethoxydim showed stress of *Digitaria* plants, but then there was an indication of temporarily recovery of these plants for 2 to 3 days. A similar rise in Fo was noted at day 5 in leaves treated with all dose rates of sethoxydim with exception of the full dose rate (579 g a.i.ha^{-1}). But the fluorescence (Fo) values obtained from the plants treated with this dose rate were still high compared to those obtained from the untreated plants. Increase of fluorescence in susceptible plants treated with sethoxydim has been reported elsewhere (Richard *et al.*, 1983; Hubbard and Whitwell, 1991). The present study indicated that fluorescence (Fo) in leaves treated with sethoxydim was low for all dose rates at day 14, 21 and 28, as it was also observed for fluazifop-butyl. Sethoxydim, as well had significant effect on the Fv/Fm in the treated leaves of *D. abyssinica* compared to the untreated. Reduction in Fv/Fm was noted for all sethoxydim concentrations compared to the control shortly after treatment, and this can be associated with the rise of Fo at the time (Figure 3.9). It was however noted that there was an increase of Fv/Fm values from day 1 to 4 (0.70-0.75). The values obtained at these times were not significantly different from those obtained in the control (0.73-0.77). This is also reflected in the Fo values which were low at these times. These results tend to suggest that the plants might have temporarily recovered from the herbicide stress. Partial recovery of plants after herbicide applications has been reported elsewhere by Van Oorschot and VanLeeuwen, (1992).

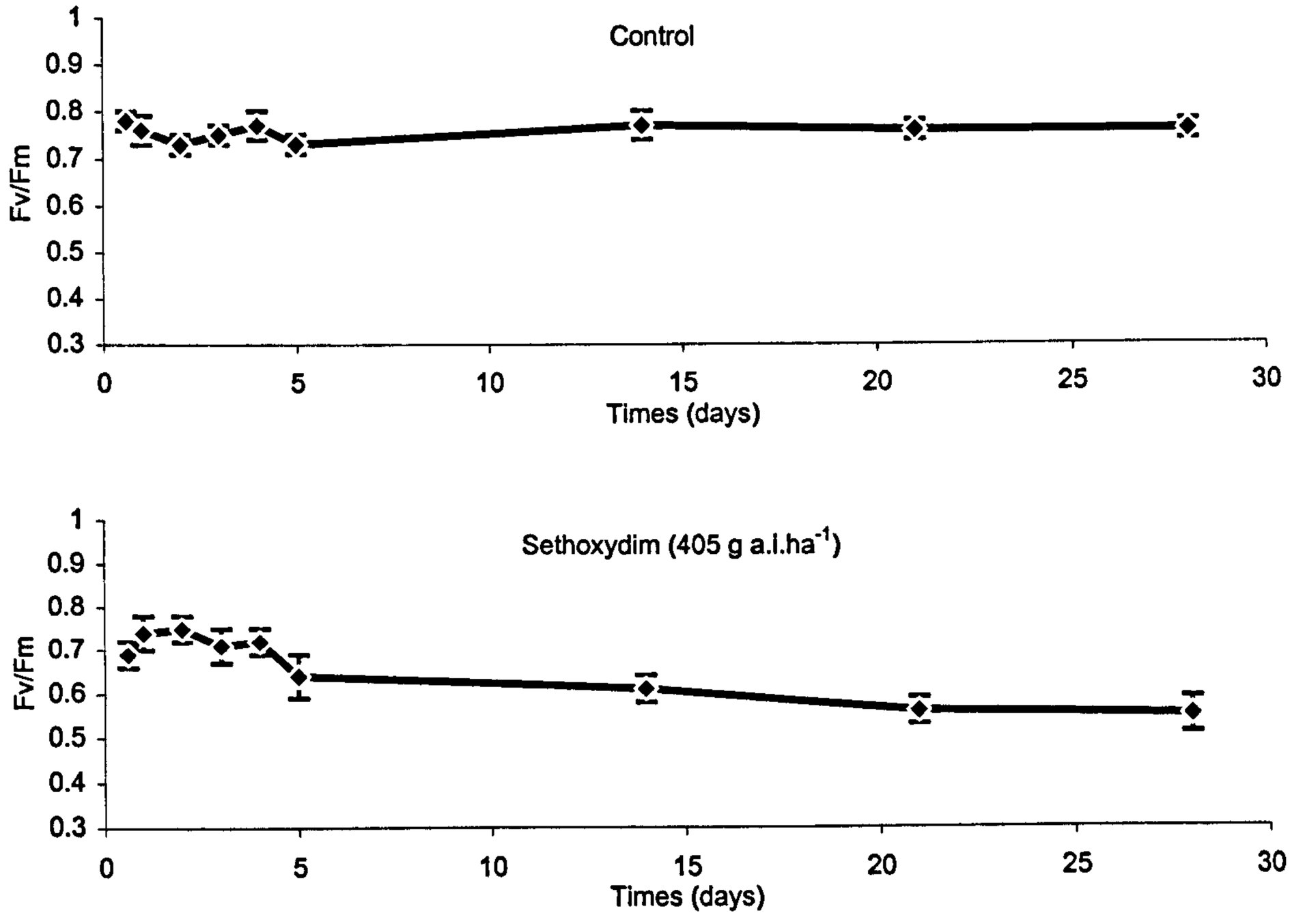
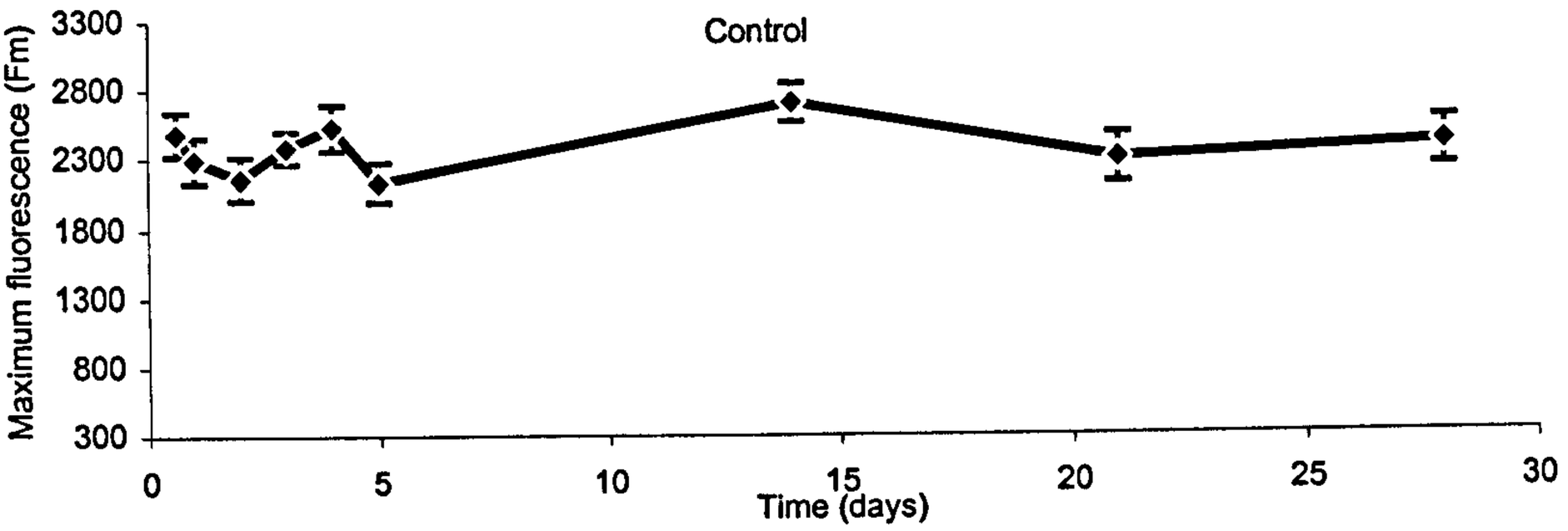
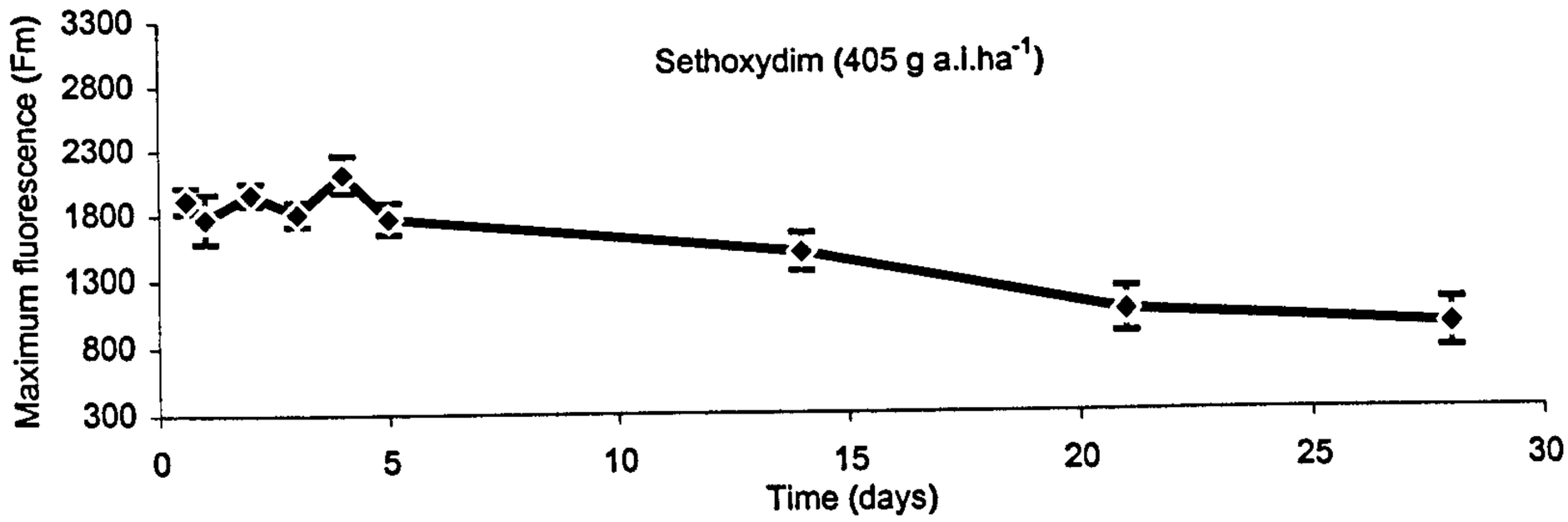
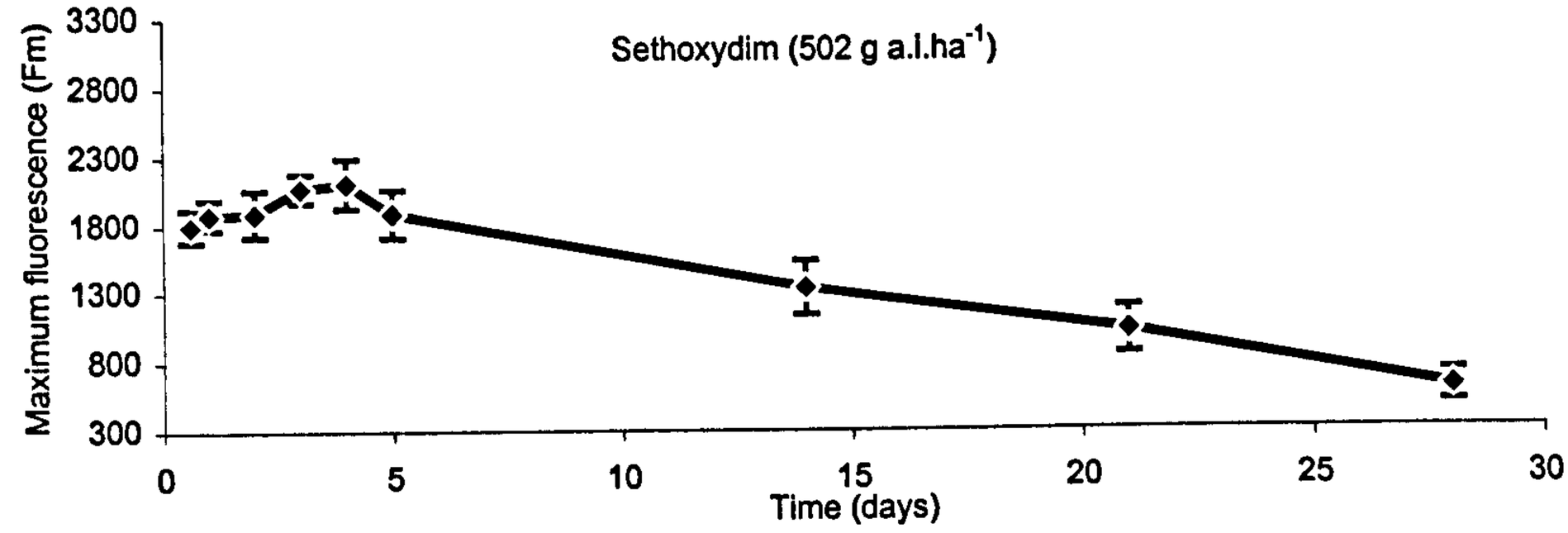


Figure 3.9.Fv/Fm of *D. abyssinica* leaves taken at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of six replications.

It was also noted that, although Fo did not rise at later periods, Fv/Fm was reduced. A similar observation was noted for fluazifop-butyl. As already mentioned earlier, fluorescence maximum (Fm) may be associated with the decrease of Fv/Fm since values of Fm are higher than those obtained for Fo (Figure 3.10). According to Richard *et al.*, (1983), increase in fluorescence maximum (Fm) occurs if excitation energy is inhibited from being utilised for photochemistry. They further explained that fluorescence maximal (Fm) can be used as a sensitive and consistent parameter for assessing inhibition of photosynthetic electron transport. In the present study results associated the increase in Fo and Fm with the decrease of Fv/Fm for both fluazifop-butyl and sethoxydim. Another observation noted was that treated leaves seemed to have started responding to both herbicides at day 5 where fluorescence (Fo) increased in almost all dose rates of both herbicides. These results could have supported the visual symptoms observed on *D. abyssinica* in the greenhouse where chlorosis of young leaves was noted within 5-7 days after treatment. Chandrasena and Sagar (1987) noted similar observations for fluazifop-butyl and Asare-Boamah and Fletcher, (1983); Hosaka *et al.*, (1984) for sethoxydim. The present study revealed that although fluorescence (Fo) commonly rose at day 5 for both herbicides, some variations were observed amongst dose rates at different times. These variations might be due to the penetration differences of these herbicides, and the rate at which they reach their site of action after absorption (Richard *et al.*, 1983).







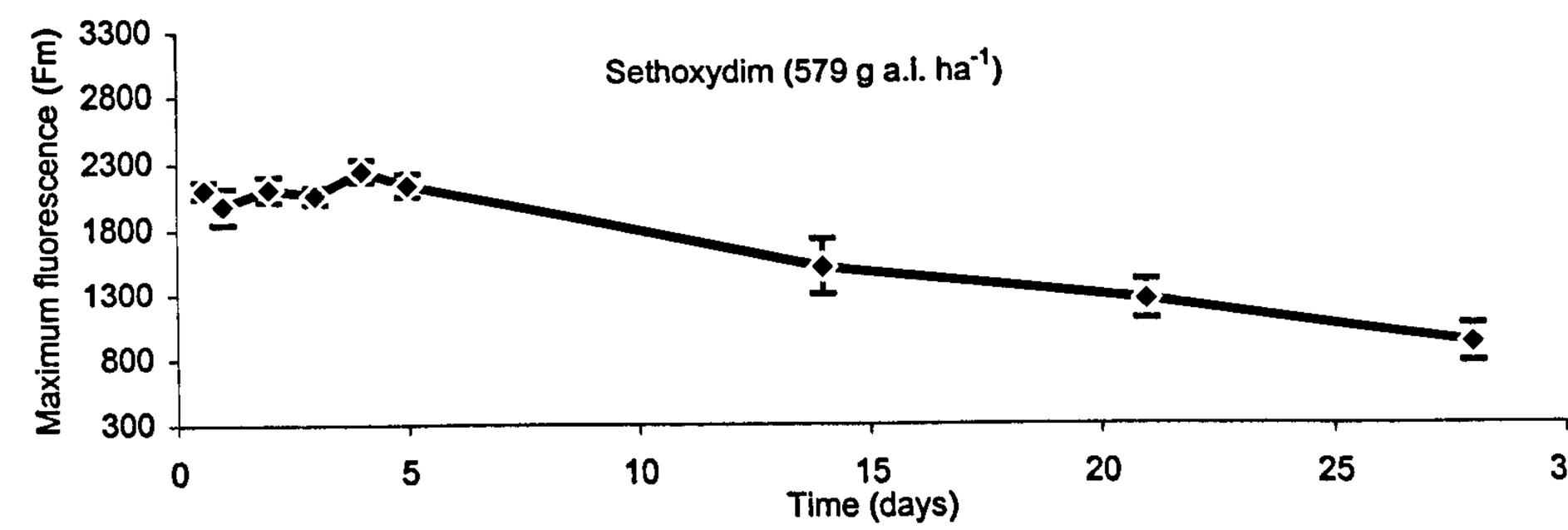


Figure 3.10. Maximum fluorescence (Fm) of *D. abyssinica* leaves measured at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of six replications.

Fluorescence of *D. abyssinica* after herbicide application was also studied in the field. The results are presented in Table 3.1. It was noted that minimum fluorescence (Fo) in the treated leaves significantly increased compared to the Fo obtained from the untreated (Appendix 3.5). These results have supported the results obtained in the greenhouse, indicating the effect of both herbicides on *D. abyssinic*a under both conditions. Similarly Fv/Fm was significantly reduced in the treated *D. abyssinica* leaves (Appendix 3.6). The values obtain on Fm were relatively high in treated leaves compared to the untreated, although they never significantly differed. Observations found that the dose rates used did not show significant differences in the reduction of Fv/Fm. This was observed for both herbicides, which is an indication that the activity of reduced dose rates against *D. abyssinica* was as good as the full dose rate activity.

Table 3.1. Fluorescence parameters measured from the treated and untreated leaves of *D. abyssinica* 21 days after herbicide application in the cotton field.

Treatment	Minimum Fluorescence (Fo)	Fv/Fm ratio	Maximum fluorescence (Fm)
Fluazifop-butyl 138 g a.i.ha^{-1}	693.5±54.2a	0.47±0.01a	1298.3±337.4a
Fluazifop-butyl 162 g a.i.ha^{-1}	661.0±38.2a	0.40±0.03a	1110.8±18.5a
Fluazifop-butyl 188 g a.i ha^{-1}	811.3±47.4a	0.52±0.05a	1708.5±318.1a
Sethoxydim 425 g a.i.ha^{-1}	571.3±23.8a	0.57±0.03a	1331.2±121.8a
Sethoxydim 501 g a.i.ha^{-1}	578.5±40.1a	0.34±0.03a	873.3±45.7a
Sethoxydim 579 g.a.ha^{-1}	640.0±14.0a	0.42±0.06a	1115.7±127.7a
Control	253.5±30.6b	0.77±0.02b	1120.5±77.7a
Sgnificant level	**	**	ns

ns- not significant **- significant at 1%, *- significant at 5%
± - represents the standard error for each mean value of four replications

CHAPTER FOUR

Response of *D.abyssinica* to fluazifop-butyl and sethoxydim applied at dose rates that are below half of the recommended dose rates.

4.1 Introduction

Use of reduced herbicide levels has been investigated following environmental concerns and to reduce crop production costs (Edwards, 1987; Steckel *et al*, 1990). Good weed control with reduced herbicide dose rates has been extensively studied (DeFelice *et al*, 1989; Muyonga *et al,* 1996). Similar observations were noted from the field and greenhouse experiments for the control of *D. abyssinica* with fluazifop-butyl and sethoxydim. However, the reduced dose rates investigated in these experiments did not significantly differ from each other in most of the parameters measured. This led to the initiation of evaluating fluazifop-butyl and sethoxydim at levels below half of the recommended rates.

The objective was

1. to evaluate association between plant stress and dose rates that are reduced below the full rates of fluazifop-butyl and sethoxydim.
2. to determine plant stress by measuring chlorophyll content and fluorescence yield of *D. abyssinica* leaves after herbicide application.

4.2 Materials and Methods

The experiment was conducted at CloseHouse field station, Heddon on the Wall, Northumberland (UK) in August 1999. *D. abyssinica* was multiplied as described in

Chapter 3. Procedures of applying the herbicides in the greenhouse were done as described in Chapter 3. This experiment was laid out in a randomised complete design (RCD) in four replications. Treatments are described in the table below;

Table 4.1 Application of dose rates reduced by more than a half of the recommended rates of fluazifop-butyl and sethoxydim for the control of *D. abyssinica*.

Number	Treatment	Dose rates (g a.i.ha^{-1})	Proportion of full rate
1	Fluazifop-butyl (Fusilade)	38	20%
2	Fluazifop-butyl (Fusilade)	66	35%
3	Fluazifop-butyl (Fusilade)	94	50%
4	Fluazifop-butyl (Fusilade)	188	Full
5	Sethoxydim (Checkmate)	116	20%
6	Sethoxydim (Checkmate)	203	35%
7	Sethoxydim (Checkmate)	289	50%
8	Sethoxydim (Checkmate)	579	Full
9	Control	0	0

The above dose rates were obtained by reducing the recommended dose rate of each herbicide by 50, 65 and 80%.

4.2.1 Fresh and dry weight measurements

Fresh weight of shoots and rhizomes of *D. abyssinica* were measured 31 days after the application of the herbicides. The shoots were excised, weighed and placed in small aluminium trays. Similarly the rhizomes were harvested.

The soil was washed off rhizomes prior to weighing and they were also placed in the aluminium trays. Thereafter all the samples were placed in the oven at 90°C for 48 hours. Fresh and dry weights were determined as described in Chapter 3, Section 3.2.1.

4.2.2 Chlorophyll measurements

Chlorophyll content of *D. abyssinica* after herbicide application was measured by using a chlorophyll meter SPAD-502, Minolta Camera Co., Ltd. In this experiment, chlorophyll content was measured at the end of the experiment because the chlorophyll Meter was not available during the experimentation period. Thus data was not recorded at different times. This Chlorophyll Meter can also be used to take measurements in the field. Its measuring area is 2mm x 2mm, to allow measurements of small leaves. Thickness of the leaf samples may be up to 1.2mm. Thick leaves with a lot of veins can not be accurately measured by SPAD-502. In such cases, it is important to take several measurements and calculate the average to obtain better results. Values obtained by using SPAD-502 are equivalent to the chlorophyll content present in the leaf. These values are calculated depending on the amount of light transmitted by the leaf in two wavelength regions in which the absorbance is different.

4.2.3. Fluorescence measurements

These measurements were done at different periods after the application of the herbicides. The first measurement was done 3 hours after herbicide application. Then the subsequent measurements were taken at 1, 4, 7,10, 26, 29 and 31 days. The equipment used to

measure fluorescence, and measurement procedures are described in Chapter 3, section 3.2.2.

4.2.4. Data analysis

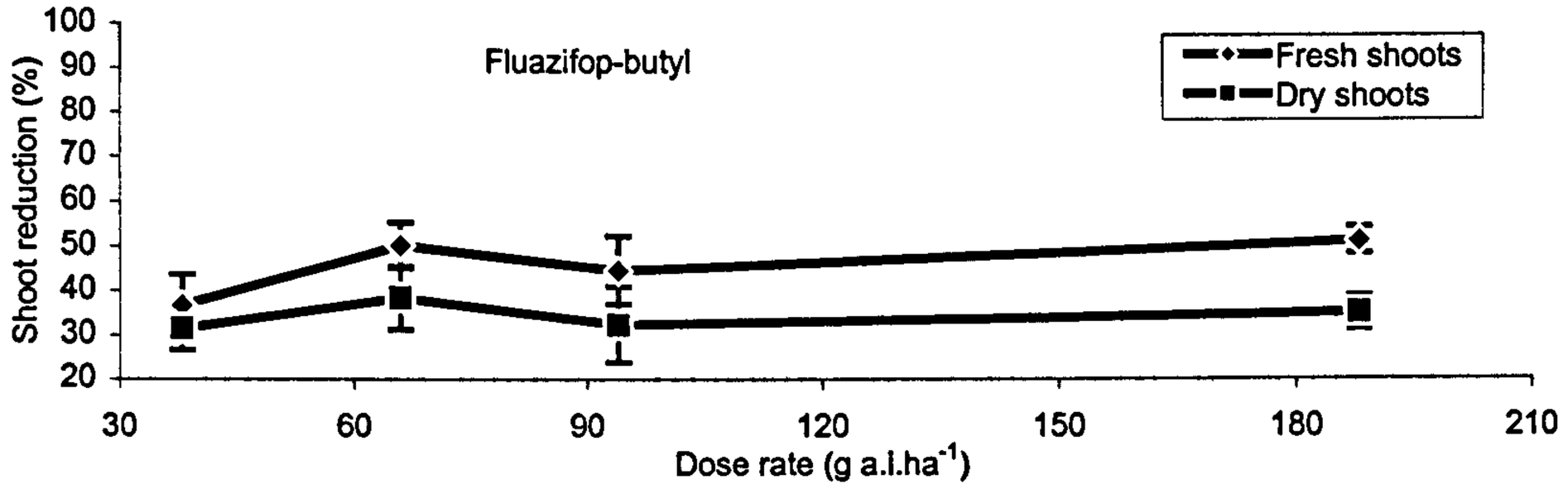
Data collected on the fluorescence parameters, chlorophyll content and fresh and dry weights of *D. abyssinica* were subjected to analysis of variance (ANOVA). The means were separated at 5% level significance using Tukey's multiple range test. As the relationship between chlorophyll content and dose was strongly curved at log (dose + 1), transformation was used to linearise the data before correlation and regression were performed.

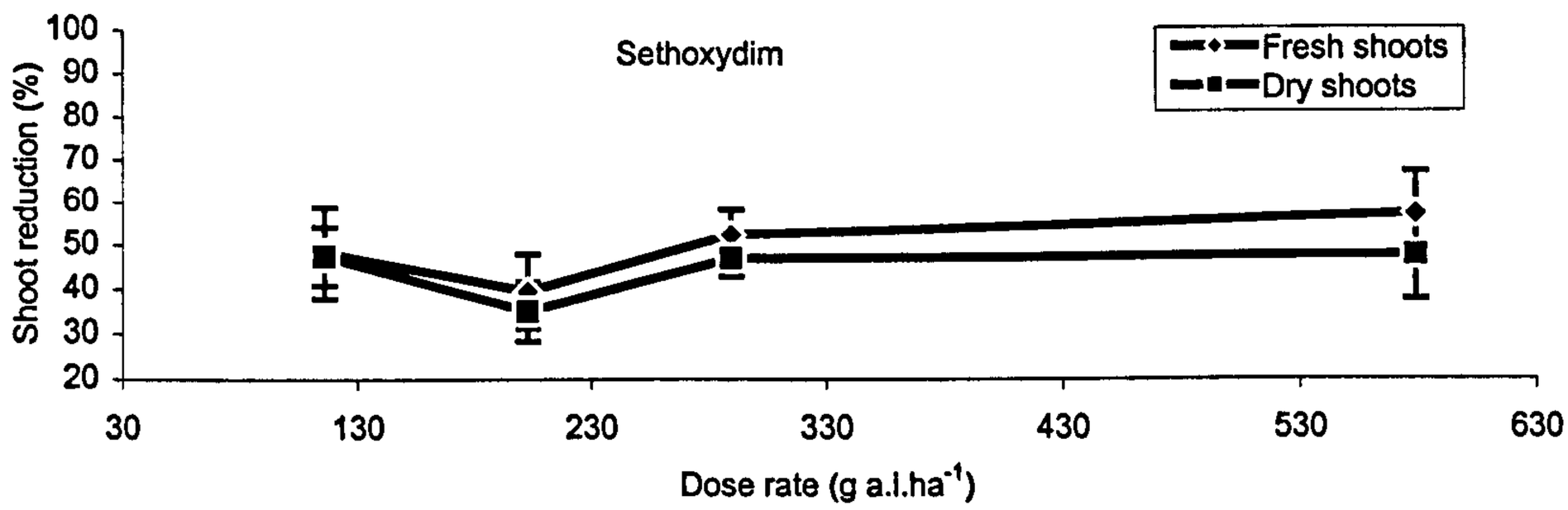
4.3 Results and Discussion

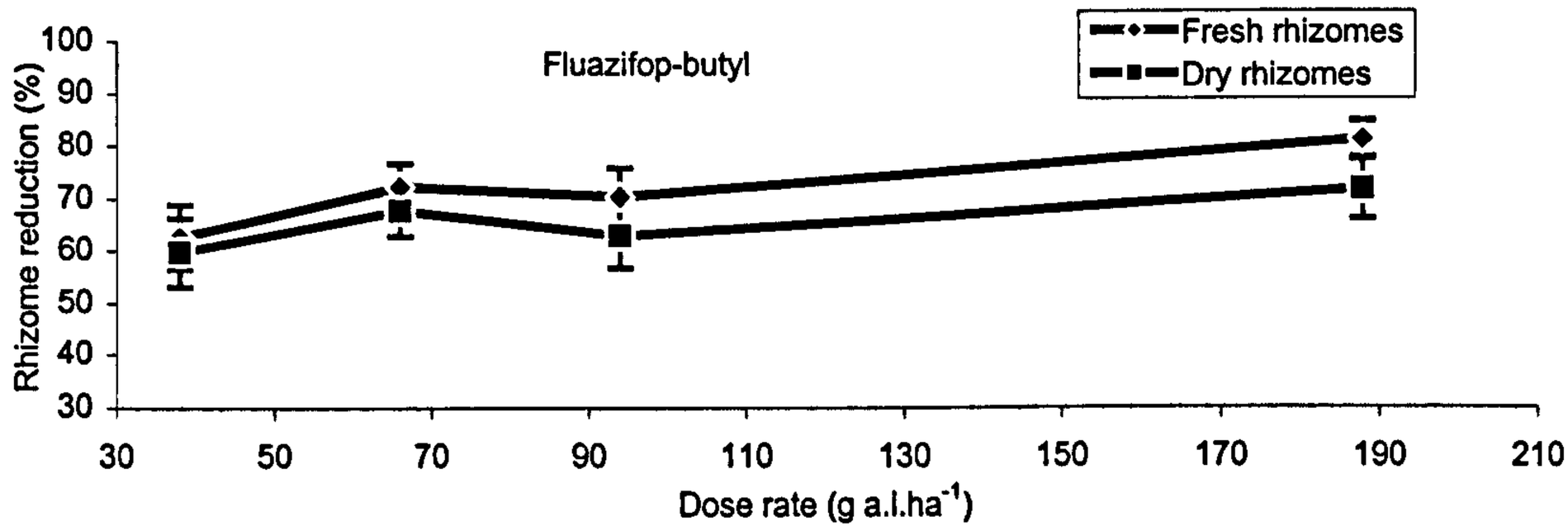
4.3.1 Activity of fluazifop-butyl and sethoxydim on the shoots and rhizomes of *D. abyssinica*

Previous studies indicated much about the high yield losses associated with *D. abyssinica* (Webb *et al.*, 1993). The weed is quite difficult to control by cultivation only due to its rhizomatous rooting system. Todate, chemical control seem to be the only solution of controlling this weed. However, there is an emphasis on the use of herbicide low levels to reduce expenses of controlling this weed and protecting the environment. The present study has revealed a lot about the control of *D. abyssinica* with sethoxydim and fluazifop-butyl at very low and high concentrations.

The results obtained in this study indicated that all herbicide levels used in this experiment, showed significant reduction of the biomass compared to the control (Figure 4.1 and Appendices 4.1, 4.2, 4.3, 4.4). Although analysis of variance showed that there were no significant differences amongst the dose rates of both herbicides, some plant regrowth was observed in pots treated with the lowest dose rates (visual observation). These lowest doses were fluazifop-butyl (38 g a.i.ha^{-1}) and sethoxydim (116 g a.i.ha^{-1}). It was also noted that these dose rates gave relatively high values of the fresh and dry weights of *D. abyssinica* compared to the other dose rates. These data have revealed the extent at which the lowest dose rates can control of *D. abyssinica*. The results probably suggest that the lowest dose rates can suppress growth and kill the main shoot but not the meristematic region, hence regrowth occurred (Smith and Vanden Born, 1991). Foliage regrowth of other weeds after the application of sublethal dose rates of various herbicides such as glyphosate has been reported elsewhere (Cole *et al.*, 1983). Results obtained in the present study on the reduction of fresh and dry weights could be evident that fluazifop-butyl and sethoxydim inhibited the growth of *D. abyssinica*. Observations found that on average, the reduction of fresh and dry shoots ranged between 30-57.6%. These results have revealed the activity of sethoxydim and fluazifop-butyl against *D. abyssinica*. The results supported results obtained by Hicks and Jordan (1984) while evaluating fluazifop-butyl and sethoxydim on various grass species. According to Harker, (1995) herbicide phytoxicity can be based on the reduction of the plant biomass. Both herbicides showed the potential of controlling *D. abyssinica*. According to Terry (1988), although fluazifop-butyl and sethoxydim have distinct chemical structures, they cause similar symptoms on most grass species.







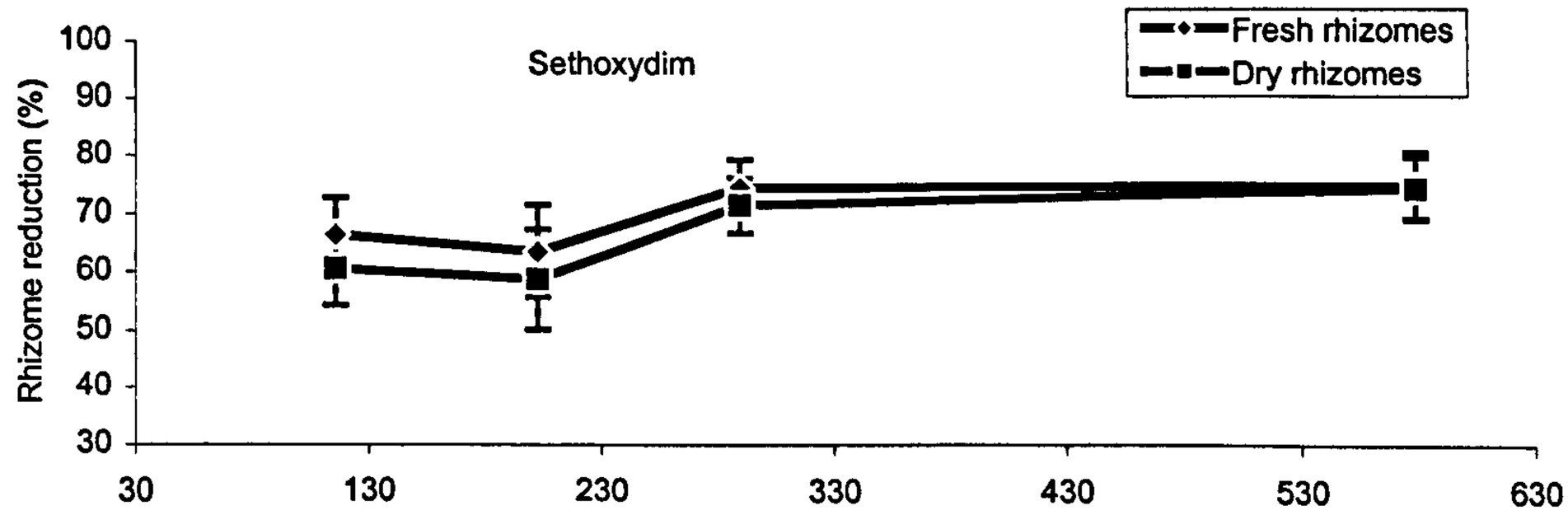


Figure 4.1.Percentage reduction of *D. abyssinica* biomass obtained after the different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of four replications.

And their similarity in herbicidal activity against most grasses was reported by Harwood *et al.*, 1989. The fresh and dry weights of the *Digitaria* rhizomes showed almost a similar trend as observed on the shoots. Both fresh and dry weights of *Digitaria* rhizomes were drastically reduced by both herbicides compared to the untreated. An average percentage reduction of 50-81.4% was noted for both fresh and dry rhizomes. Reduction of fresh and dry weight of rhizomes of other grass weed species such as johnsongrass due to fluazifop-butyl and sethoxydim has been studied (Harker and Dekker, 1988). As noted from the shoots weights, rhizomes treated with the lowest dose rates of both herbicides gave high weights. This also suggested poor control of the rhizomes with the lowest dose rates. However, Streibig, 1992 suggested that the effectiveness of herbicide low levels can be obtained in a later phase of chemical control. The present study has however, given evidence that sethoxydim and fluazifop-butyl were adequately absorbed and translocated in the plants of *D. abyssinica*. This is shown by the extent of reduction in shoot and rhizome weights, especially with the treatments of half and full dose rates of both herbicides.

4.3.2. Water content of *D. abyssinica* after herbicide application

There was a significant ($P \leq 0.01$) linear correlation between the percentage reduction of water content in the shoots and rhizomes of *D. abyssinica* and the dose rates of sethoxydim and fluazifop-butyl (Figure 4.2).

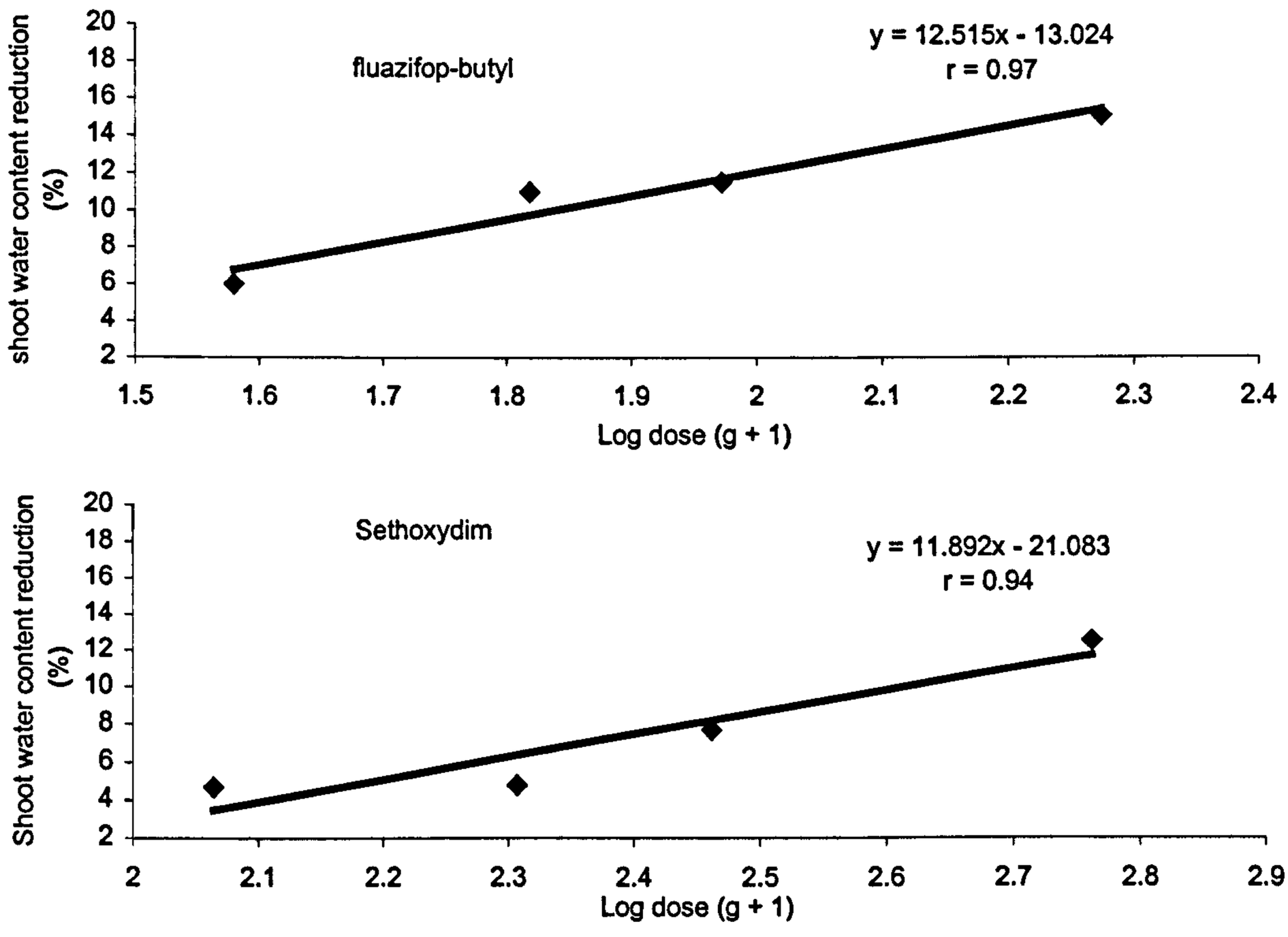
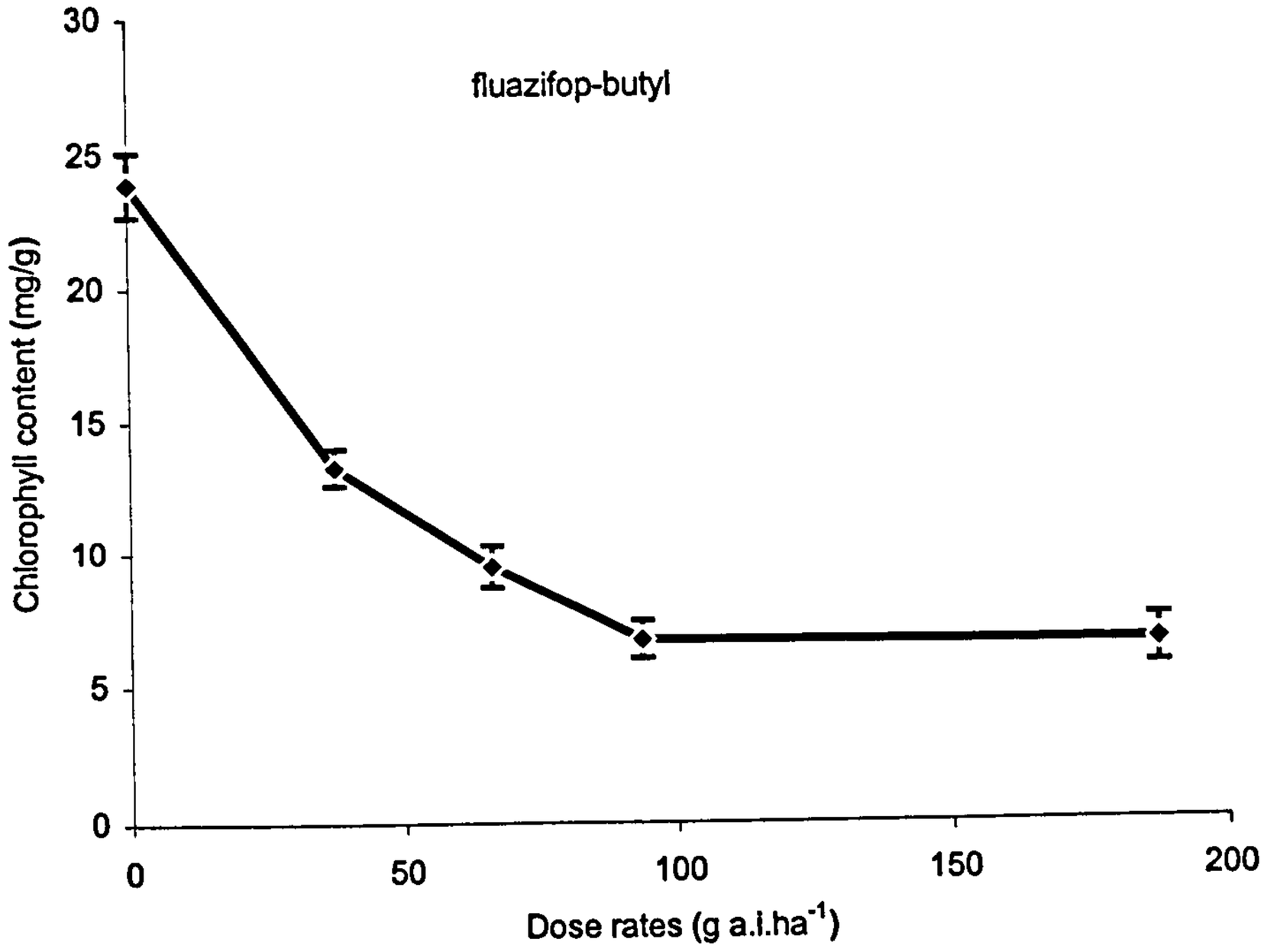


Figure 4.2.Relationship between water content reduction of the shoots and rhizomes of *D. abyssinica* and herbicide dose rates obtained from the greenhouse data.

Results seemed to suggest that water loss was dose dependent. The significant loss of water indicated plant stress as a result of herbicide activity against *D. abyssinica*, which is also reflected in the reduction of the fresh and dry weights of the weed, possibly suggesting plant growth inhibition. Determining plant injury through measuring plant water content has been studied elsewhere (Pignata *et al.*, 1999). In other studies, Pimentel *et al.*, (1990) pointed out that reduced shoot dry weight significantly indicates plant water stress.

4.3.3 Chlorophyll content in the leaves of *D.abyssinica* after herbicide application.

Loss of chlorophyll content as part of the death process of the plant can be detected from the visual symptoms caused by the herbicides after treatment (Chandrasena and Sagar, 1984). The present study showed that all dose rates tested, significantly reduced chlorophyll content in the treated leaves compared to the untreated (Figure 4.3 and Appendix 4.5). However, significant differences were also observed amongst dose rates of fluazifop-butyl, with the lowest dose rates (38 and 66 g a.i.ha^{-1}) giving high values of chlorophyll content. This could have suggested a delayed chlorophyll breakdown in the less phytotoxic levels (VanOorschot *et al.*, 1979; Kidd and James, 1991) The application of sethoxydim on *D. abyssinica* similarly showed a significant reduction in the chlorophyll content and results indicated that sethoxydim had a high percentage reduction of chlorophyll content compared to fluazifop-butyl. This could probably have been due to the rate at which sethoxydim metabolites move in the plant.



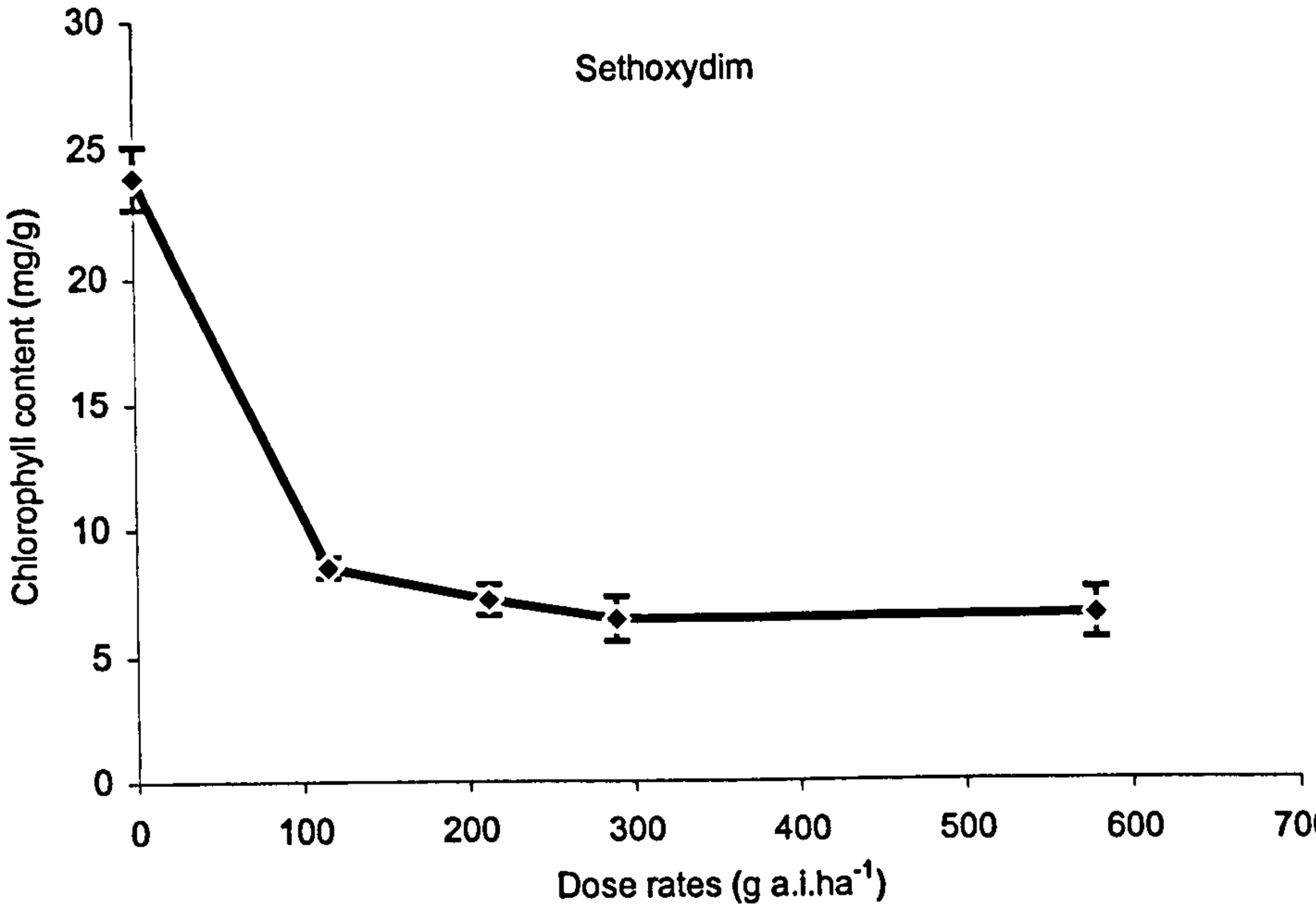
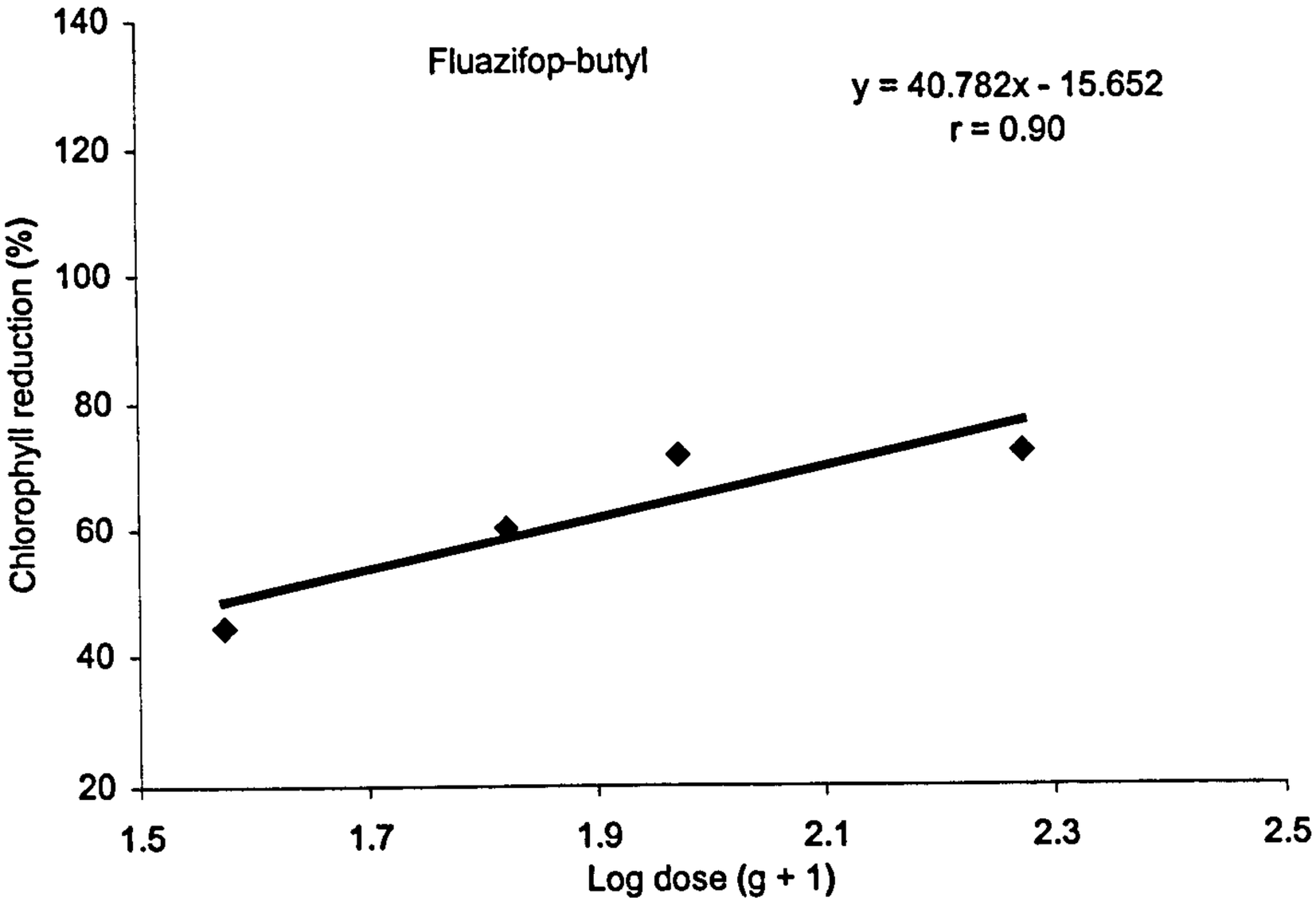


Figure 4.3. Chlorophyll content of *D. abyssinica* leaves measured after different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of four replications.

As reported by Cole, 1994 that sethoxydim metabolites move slowly in the plant and this gives them the potential to consolidate their detoxifying effect. A similar situation could have occurred when this herbicide was applied on *D. abyssinica*. In general however, the present results have given an average chlorophyll content reduction of 44-73% as a result of herbicide application. According to Campbell and Penner, (1981), the decrease in chlorophyll accumulation after herbicide treatment can be a result of chloroplast disruption or inhibition of carotenoid synthesis which might be followed by photodestruction of chlorophyll. According to Noodén *et al.*, (1997) loss of chlorophyll is another measurement to determine leaf senescence. This gives an implication that senescence of *D. abyssinica* could have partly been due to loss of chlorophyll content after the application of sethoxydim and fluazifop-butyl. In other studies, reduction of chlorophyll content due to sethoxydim and fluazifop-butyl were reported (Lichtenthaler and Kobek, 1987; Fernandez *et al.*, 1987; Magallanes *et al.*, 1986; Wakeham and Kirkwood, 1991; Hallgren and Fischer, 1992). Several other herbicides are known for affect chlorophyll breakdown (Fletcher and Kirkwood, 1982). Figure 4.4 illustrates that the relationship between percentage chlorophyll reduction and the herbicide dose rates was a strong relationship with the fluazifop-butyl traetments ($r = 0.90$, $P \leq 0.01$). A similar trend was noted from the sethoxydim treatments ($r = 0.88$, $P \leq 0.01$), though the slope was much smaller. This tends to suggest that percentage chlorophyll reduction was more dependant on the herbicide levels of fluazifop-butyl than it was with sethoxydim concentrations.



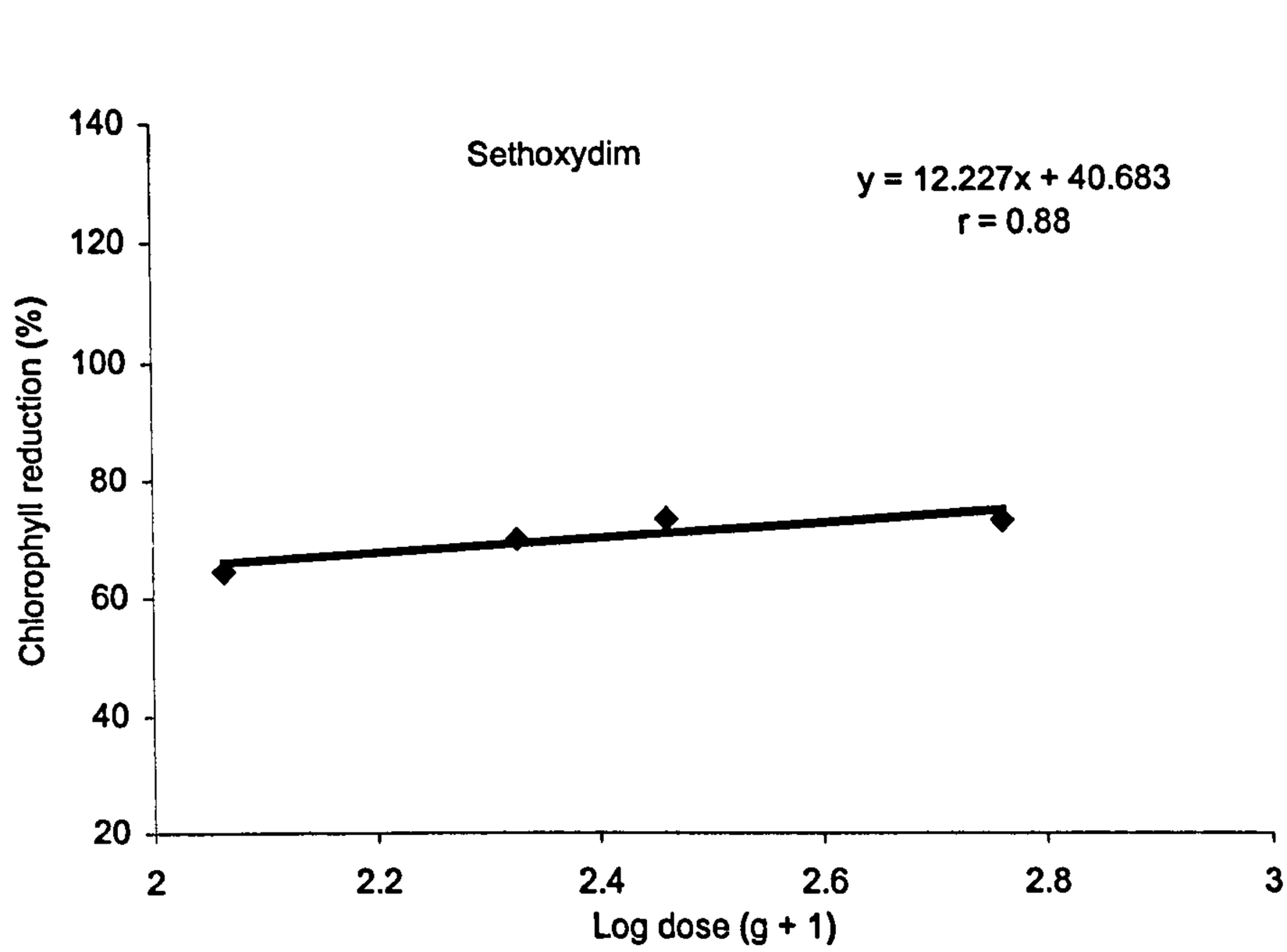


Figure 4.4. Relationship between percentage chlorophyll reduction of *D. abyssinica* leaves and herbicide dose rates obtained from the greenhouse data.

4.3.4 Fluorescence measurements after herbicide application

The fluorescence curves obtained from fluazifop-butyl and sethoxydim treated *Digitaria* leaves after leaf dark-adaptation are shown in Figures 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10. Previous studies have showed that the measurements of fluorescence can quantify the interference of herbicides with the photosynthetic electron transport (DePrado *et al.*, 1992; DePrado *et al.*, 1995 and Norsworthy *et al.*, 1998). And the dark-adaptation procedure has been reported for giving good and reliable results (Voss *et al.*, 1984 and Devine *et al.*, 1993). In the present study results indicated that the minimum fluorescence (Fo) remained relatively stable in the untreated leaves compared to the leaves treated with fluazifop-butyl (Figure 4.5). It was observed that the activity of fluazifop-butyl dose rates seemed to have started at the period of 7 days where Fo appreciably increased compared to the control which could have indicated injury of the *Digitaria* plants. This agreed with the results obtained by Chandrasena and Sagar, (1984), that plant injury symptoms (chlorosis and necrotic) due to fluazifop-butyl develop between 5-14 days. On the other hand, in a review by Maxwell and Johnson (2000), when a leaf is exposed to light from darkness, the PS II reaction centres are closed, hence this contributes to the increase of chlorophyll fluorescence.

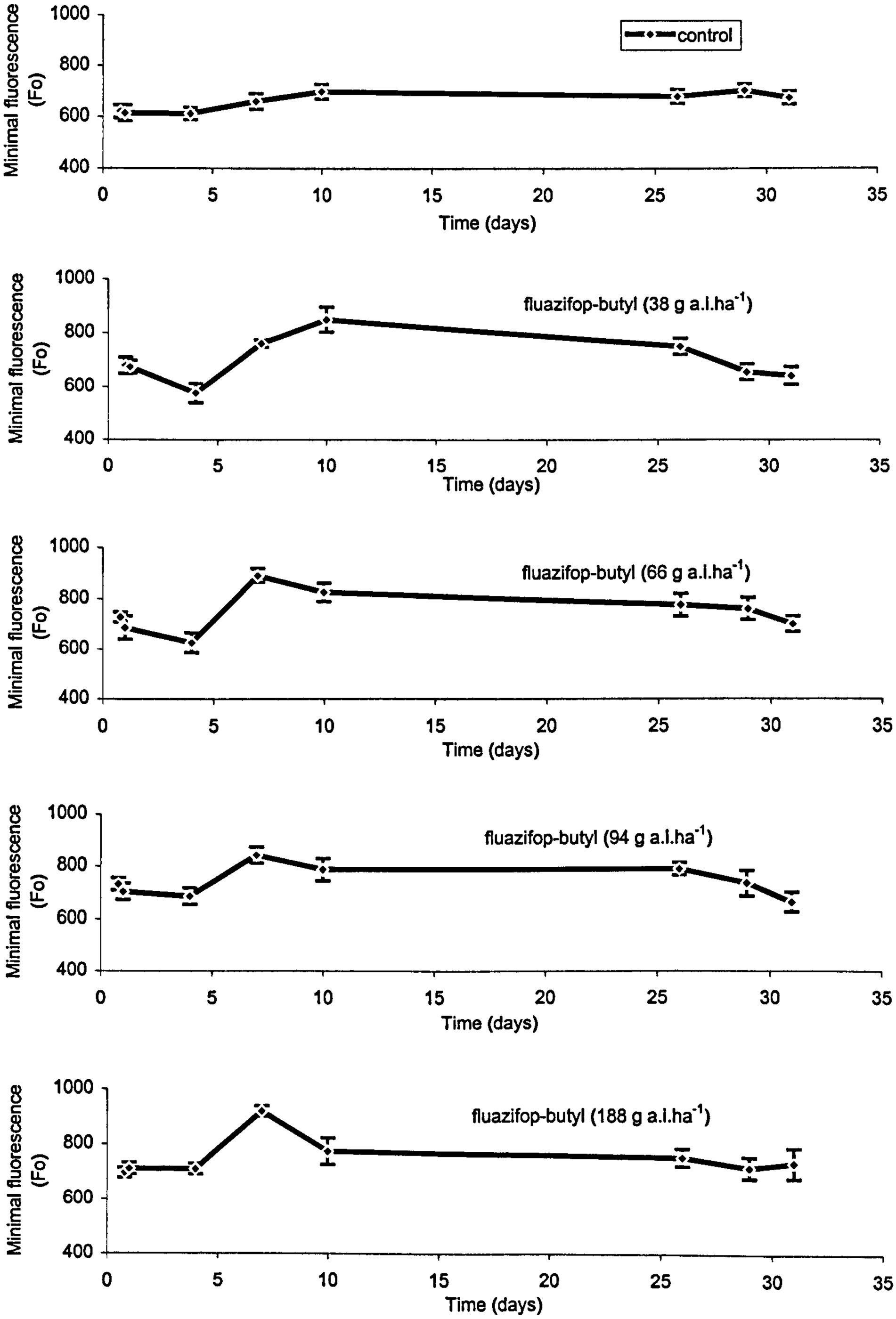


Figure 4.5.Minimum fluorescence measured from *Digitaria* leaves at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of four replications.

A similar situation could have occurred in the leaves of *D. abyssinica* after the application of the herbicide probably indicating a damage on the reaction centres. Further observations showed that unlike other dose rates, Fo markedly increased at 10 day period with the lowest dose rate of fluazifop-butyl (38 g a.i.ha^{-1}), suggesting a delay in the activity of this dose rate against *D. abyssinica*. This could have been due to its weak concentration or rather the rate at which it penetrated into the leaves. However, all dose rates of fluazifop-butyl appeared to have had an effect on the Fo of the *Digitaria* leaves. Alteration of fluorescence in susceptible *D. abyssinica* leaves might be due to PSII electron transport inhibition (Truelove and Hensley, 1982). Other herbicides such as diuron and atrazine have been reported to stimulate fluorescence rise (Izawa, 1977; Moreland, 1980). They further explained that the rise of fluorescence could be due to blockage of electron flow from electron acceptor Q_A to plastoquinone causing accumulation at Q_A. A Similar situation could have occurred in *D. abyssinica* leaves treated with fluazifop-butyl. According to Norsworthy, *et al.*, 1998, PSII consists of two electron acceptors which are responsible for fluorescence response. And these are Q_A (D-2 reaction centre) and Q_B (D-1 reaction centre protein). Fv/Fm which was also obtained through leaf dark-adaptation was reduced in fluazifop-butyl treated leaves from 7 to 31 days (Figure 4.6). This was compared with the untreated leaves which gave high values of Fv/Fm throughout the experimentation. Fv/Fm reflects the potential quantum efficiency of PSII and is used as an indicator of plant photosynthetic performance (Björkman and Demmig, 1987; Johnson *et al.*, 1993).

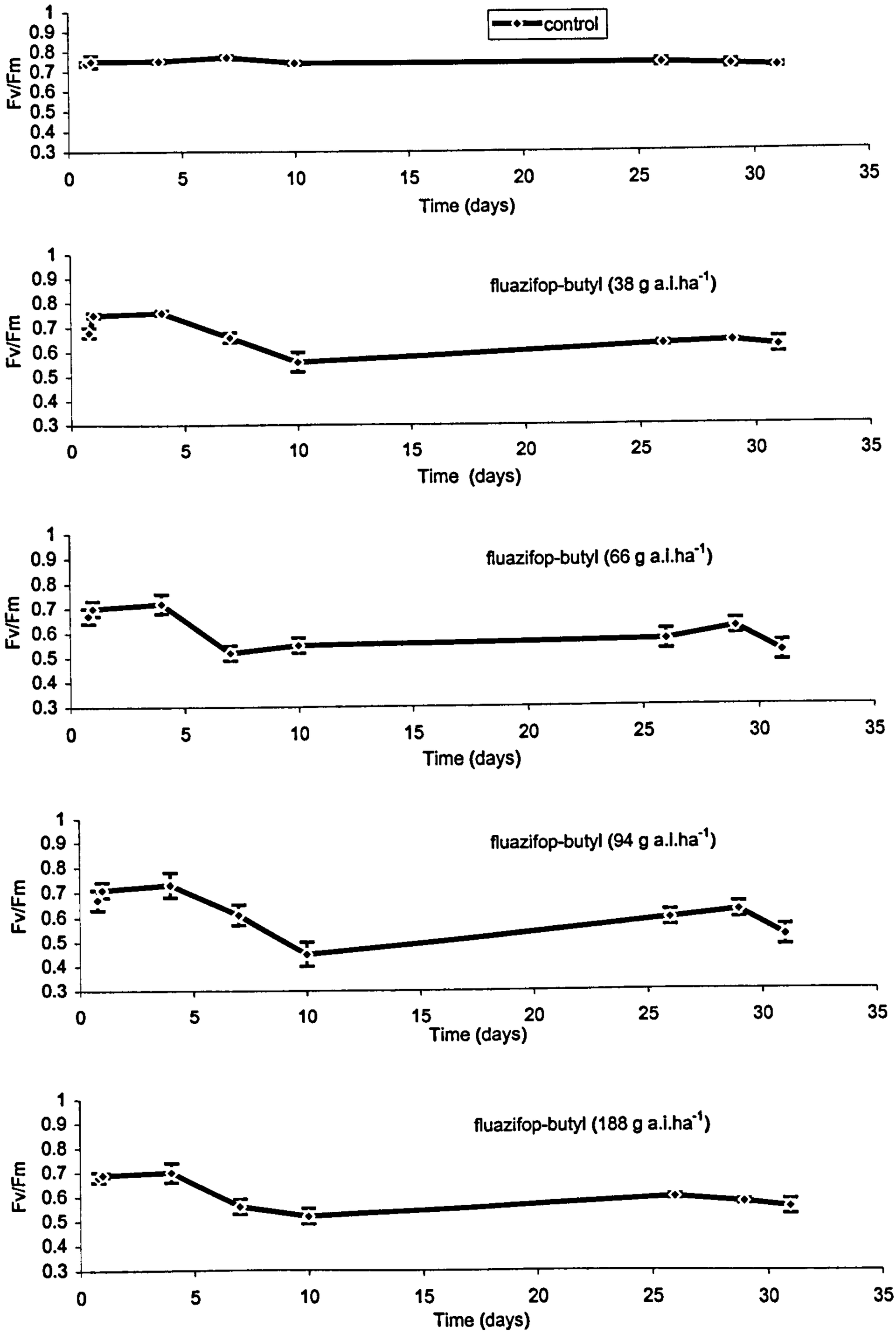


Figure 4.6.Fv/Fm of *Digitaria* leaves measured at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of four replications.

The present study showed that the reduction of Fv/Fm in the treated leaves could have been associated with the rise of the minimum fluorescence (Fo). Further observation however, found that, although minimum fluorescence (Fo) did not remarkably rise from 10 to 31 days, Fv/Fm was reduced. Then the decrease in Fv/Fm can be associated with maximal fluorescence (Fm) which was relatively high (Figure 4.7), since Fm relative values are always related to the photochemical quenching (Maxwell and Johnson, 2000). It might then imply that reduction of Fv/Fm in the treated *Digitaria* leaves could have indicated further disruption of the normal fluorescence quenching. Fluazifop-butyl dose rates did not significantly ($P \geq 0.05$) differ from each other in the reduction of Fv/Fm, although lowest rates (38 and 66 g $a.i.ha^{-1}$) gave relatively high values (0.56 and 0.58) compared to 94 and 188 g $a.i.ha^{-1}$ (0.45 and 0.52) at day 10. Figure 4.8 represents sethoxydim. treatments which similarly indicated fluctuation of the minimal fluorescence (Fo) in the treated *Digitaria* leaves recorded at different times. But the minimum fluorescence of the untreated leaves remained relatively stable. Observations found that the increase of the minimum fluorescence was commonly obtained between days 7 and 10 for most dose rates. This could have also indicated the period at which the herbicide injured the *Digitaria* plants and as a result fluorescence quenching was affected at these times, and therefore this can be used as a logical result of the herbicide toxicity (Hubbard and Whitewell, 1991). Fo was noted rising again at 26 for almost all dose rates and later decreased at day 31.

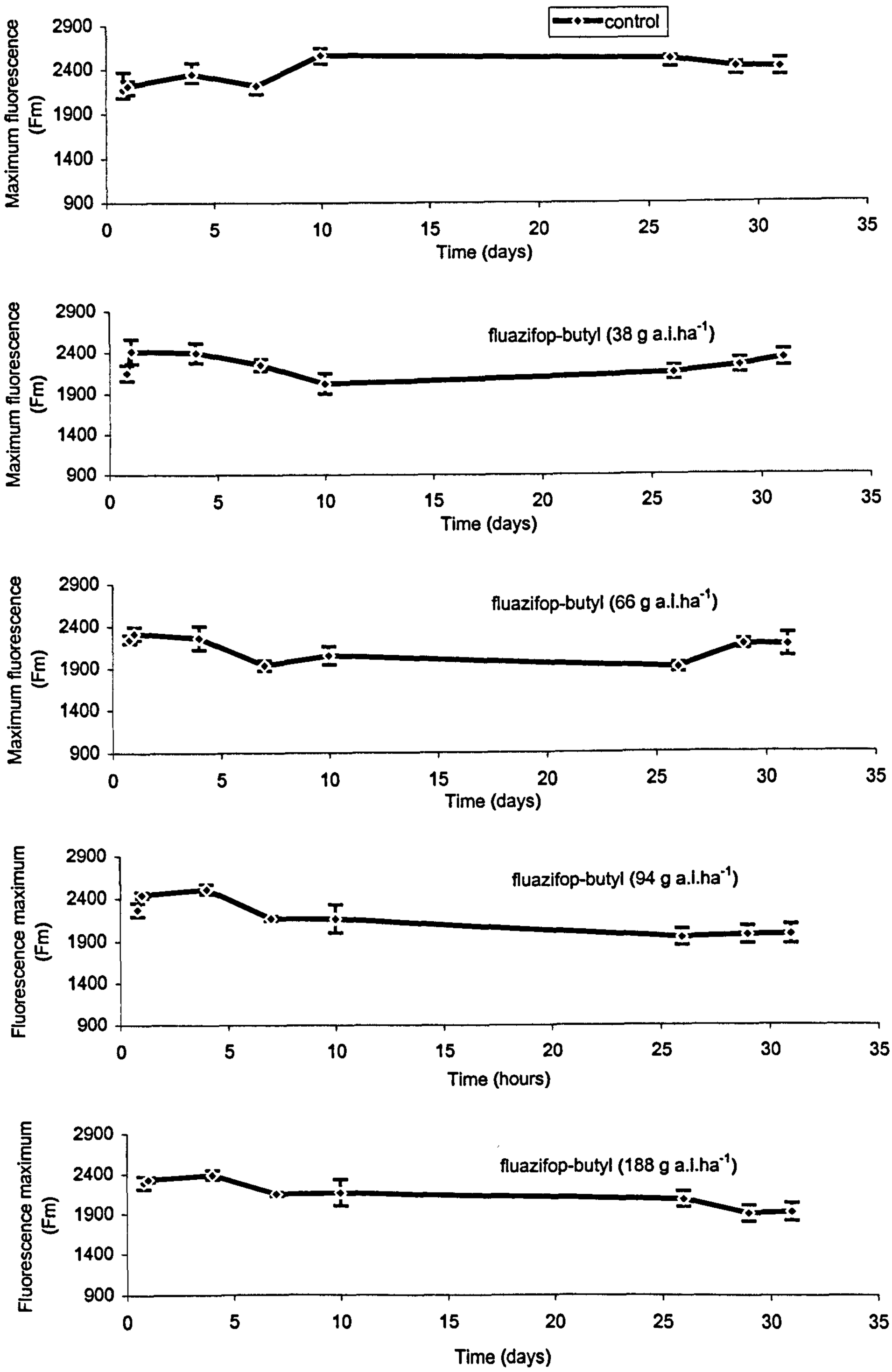


Figure 4.7.Maximum fluorescence of *Digitaria* leaves measured at times after different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of four replications.

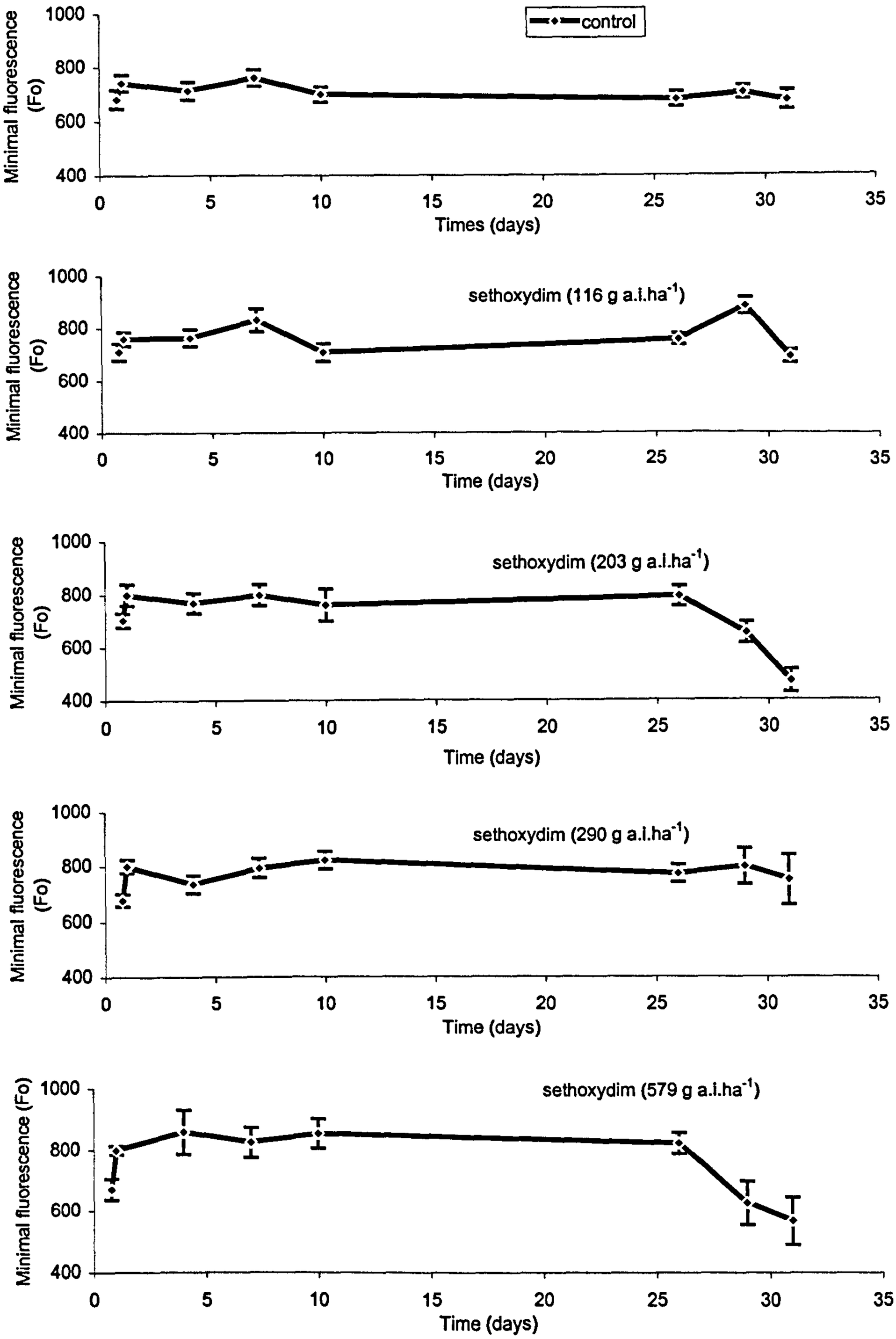


Figure 4.8.Minimal fluorescence of *D. abyssinica* leaves obtained at times after different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of four replications.

A similar observation was noted for fluazifop-butyl dose rates. Another observation found that there was some increase of Fo from days 0.8 to 1 and thereafter it decreased. A similar observation was noted in the results obtained in Chapter 3, which indicated temporarily plant recovery. Recovering of plants from herbicide stress has been reported elsewhere (Hoppe, 1980). Fv/Fm values of the *Digitaria* leaves treated with sethoxydim was significantly ($P \leq 0.05$) reduced at day 7 and 10 (Figure 4.9). The observation was also associated with the rise of fluorescence at these periods. Results indicated that although fluorescence rose at 26 and 29 days in the lowest dose rates, Fv/Fm was not as low as at 7 and 10 days. This probably indicated that there was no further disruption in fluorescence with these weak dose rates. This is reflected in the regrowth which was observed towards the end of the experiment in pots treated with these lowest dose rates. On the other hand fluctuation in the activity of the herbicide dose rates at different times might be due to the active metabolites present at each period. Results obtained by Swisher and Corbin, 1982 revealed that certain metabolites decrease or increase with time after herbicide application. Further observation in the present study revealed that Fv/Fm was markedly reduced in plants treated with sethoxydim at 289 and 579 g a.i.ha^{-1} irrespective of the low fluorescence minimal (Fo) at later periods. Similar observation was noted in the results obtained from Chapter 3. This might another indication that the maximum fluorescence (Fm) influenced plant stress (Figure 4.10). The herbicide could have inhibited fluorescence quenching and as a result had an effect on the photosynthetic photochemistry.

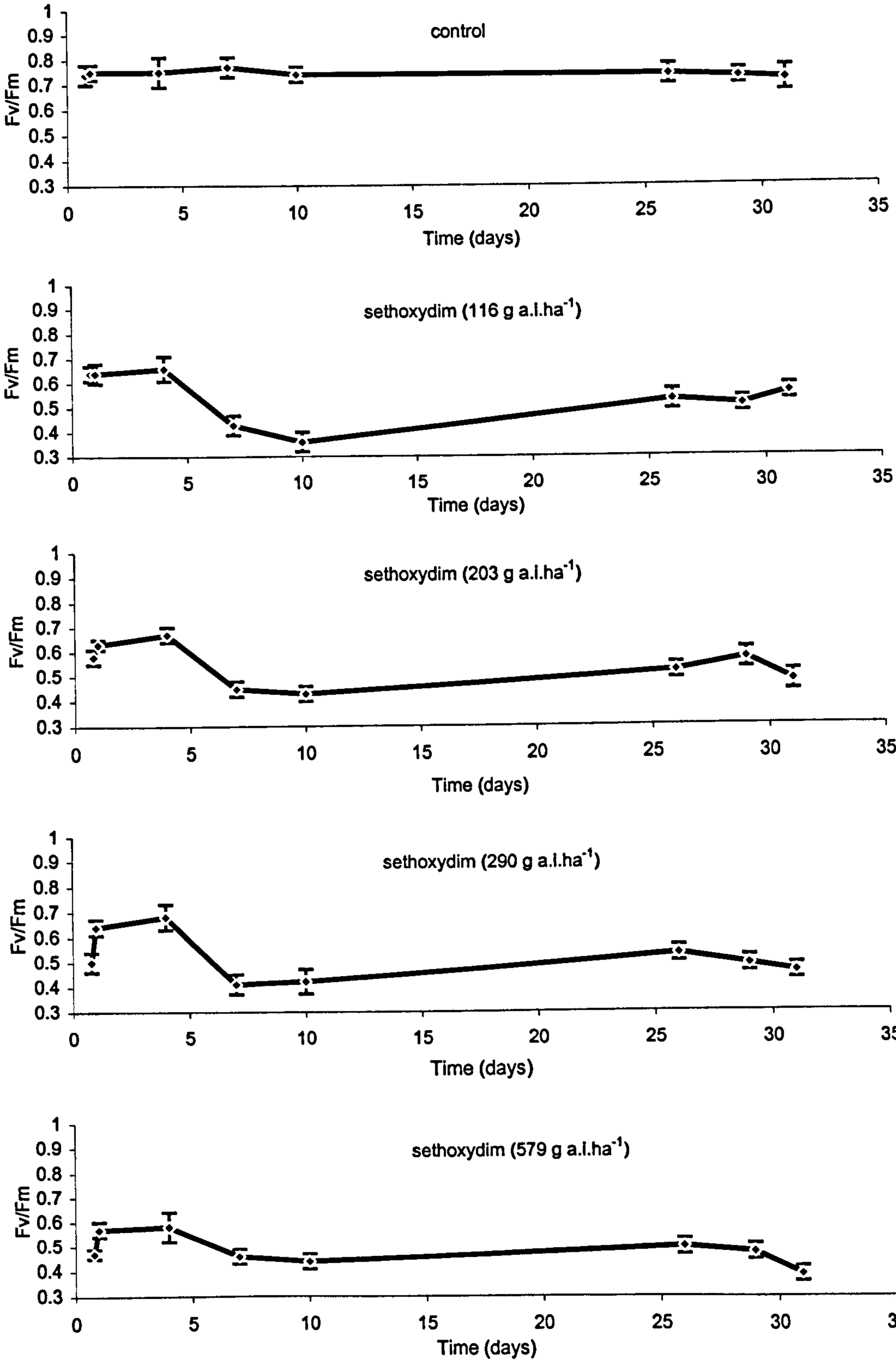


Figure 4.9.Fv/Fm of *Digitaria* leaves measured at times after different levels of herbicide application in the greenhouse.
Bars represent standard error of each mean value of four replications.

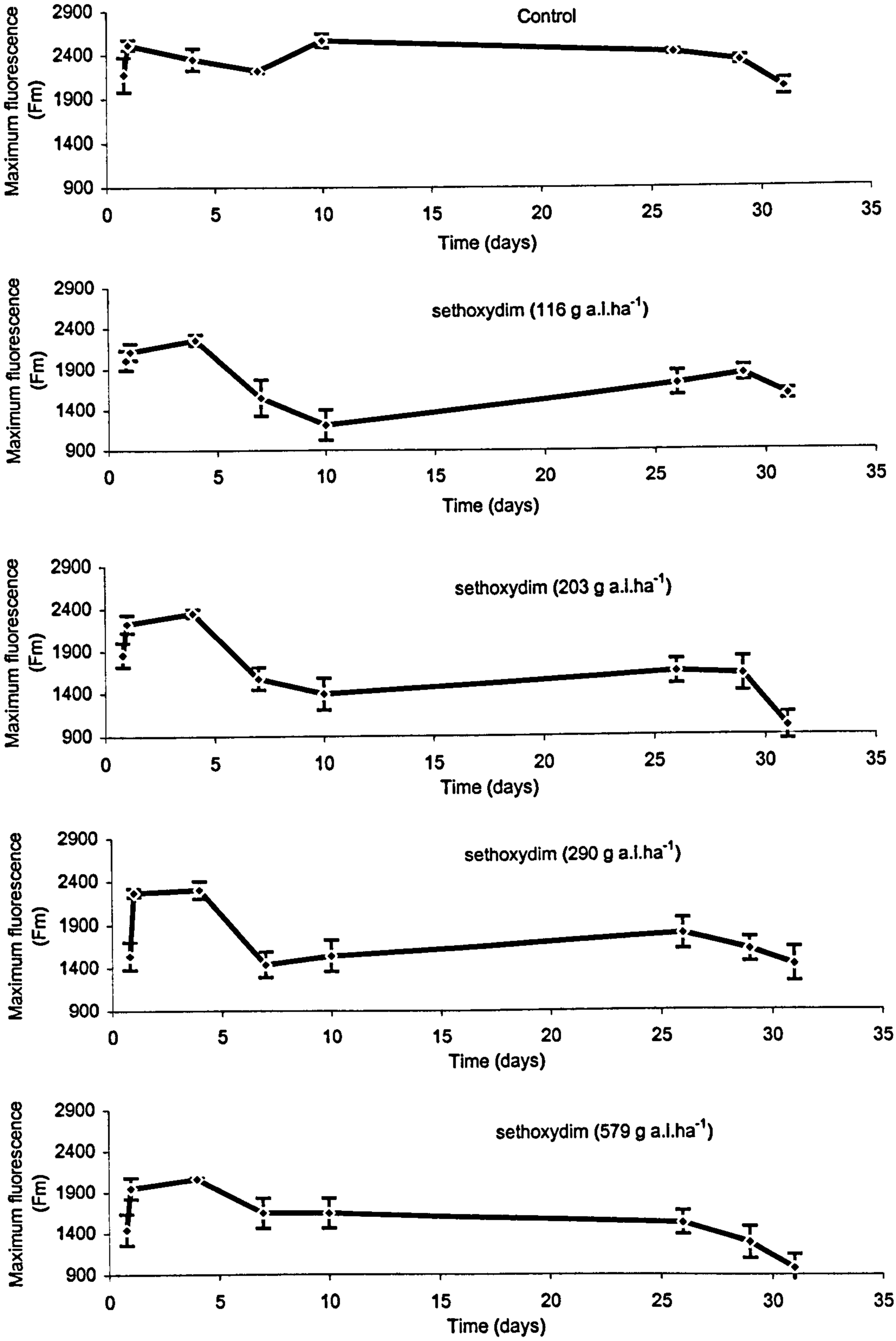


Figure 4.10.Maximum fluorescence of the *Digitaria* leaves obtained at times after different levels of herbicide application in the greenhouse. Bars represent standard error of each mean value of four replications.

Similar observations have been reported for other grass species such as *Elymus repens* and *Calamagrostis arundinacea* (Richard *et al.*, 1983; Chandrasena and Sagar, 1987; Hubbard and Whitwell, 1991). It was evident that although both herbicides reduced Fv/Fm, sethoxydim gave the lowest Fv/Fm values compared to the Fv/Fm values obtained from fluazifop-butyl treatments. These results probably suggested that these herbicides differed in their activity against *D. abyssinica*, especially in the interference with electron transport. And this could have been due to the rapid metabolism of sethoxydim where the parent herbicide is degraded within 6-72h after treatment (Campbell and Penner, 1985; Hosaka *et al.*, 1987; Ishihara *et al.*, 1988). According to Campbell and Penner, (1985), some grass species such as *Echinochloa crus-galli* rapidly produce metabolites after treatment which have similar toxicity to sethoxydim, therefore these metabolites are suggested to be responsible for the enhancement of the herbicide phytoxicity.

CHAPTER FIVE

The activity of intracellular proteases in *D. abyssinica* and cotton plants in response to sethoxydim.

5.1. Introduction

5.1.1. Functions of proteases in plants

Proteases are enzymes that play an important role in the degradation of proteins through hydrolysing peptide bonds. These proteases (EC 3.4) are subdivided into peptide hydrolysing exopeptidases (EC 3.2.4 11-19) and protein hydrolysing endoproteinases (EC 3.4 21-24). Proteases are also classified according to pH optimum activity and intracellular localisation. According to Vierstra, (1996) proteases are ubiquitous in nature and are responsible for cellular housekeeping and stress response by removing abnormal/misfolded proteins, for supplying amino acids needed to synthesis new proteins, for controlling metabolism, homeosis and development by modulating the levels of key enzymes and regulatory proteins, as well as the programmed cell death of specific plant organs or cells. Proteases therefore contribute to a number of plant physiological processes such as germination, senescence and environmental stress response (Callis, 1995; Vierstra, 1996; Clarke, 1999). Proteolytic enzymes in animals, plants and micro organisms are responsible for the overall process essential to the normal functioning of all cells (Ryan and Walker-Simmons, 1981). Intracellular proteins which undergo degradation may be re-utilised by either the organism that produced them, or other organisms in order to support the functioning of new life processes. Peoples and Dalling,

(1988); Huffaker, (1990); Feller and Fischer, (1994) correlated the total proteolytic activity from crude extracts of senescing leaves with the loss of total leaf proteins. Other studies have shown an increase of proteolytic activity with the progress of leaf senescence of various plant species such as *Cyperus rotundus* (Fischer *et al.*, 1998 and parsley (Jiang *et al.*, 1999). Research elsewhere indicated that activation of cysteine proteases is associated with the programmed cell death (PCD) of animal cells (Earnshaw, 1995; Martin and Green, 1995). A similar observation was found in the plant cells of *Zinnia elegans* (Minami and Fukuda, 1995; Ye and Vamer, 1996). Intracellular protein turnover (synthesis/degradation) have been investigated. Sloan and Camper, (1986), observed a change in protein synthesis in the presence of metolachlor. In another study however, McFarland and Hess, 1983, reported that alachlor did not affect protein synthesis in oat. A similar observation was noted in carrot cells following the application of chloroacetamides (Owen *et al.*, 1983). In another study, it was noted that high levels of free amino acids were found in herbicide-induced pathogen resistance in tomato plants, although it was not clear whether the increase of the amino acids resulted from *de novo* synthesis or increased proteolysis. On the other hand it was reported that the application of herbicides to plants disturbs protease activity depending on the plant species, plant growth stage, period after treatment, and the type of herbicide (Tonecki, 1975a; Tonecki, 1975b). Protease activity in rice (*Oryza sativa*) and barnyard grass (*Echninochloa crus-galli*), following the application of thiobencarb and butachlor was studied by Kumar and Prakasii (1994). Other herbicides such as 2,4,5-T, dalapon, atrazine and bromoxynil were

also observed to affect the enzymatic activity of proteases in various plant species (Tonecki, 1975a; Tonecki, 1975b; Hagemann, 1984). Although there is evidence that herbicides are involved in the stimulation or inhibition of intracellular proteases activity in plants (Hsu and Camper, 1979; Kumar and Prakasii, 1994), little is known about the effects on protease activities. The present study was initiated to investigate the activity levels of protease in *D. abyssinica* and cotton plants, with emphasis on plant stress due to the herbicide (sethoxydim), so as to determine whether these enzymes play a role in the development of herbicide resistance/susceptibility in these plant species.

5.1.2. Objective of the study

1) to compare the activity levels of intracellular protease in *D. abyssinica* and cotton plants in response to sethoxydim.

5.2. Materials and methods

The experiment described below was conducted in the greenhouse at the University Experimental at Close House in September 1999 (UK). It comprised of two replications and two treatments, sethoxydim was applied at 579 g a.i.ha^{-1} plus the control in a complete randomised design (CRD). *D. abyssinica* was propagated in the greenhouse as described in Chapter three, section 3.2. Cotton seeds were planted in pots of 13 cm diameter. Five seeds were planted per pot. Six days after germination, the seedlings were thinned to two plants per pot at a height of 10 cm.

Sethoxydim at the above dose rate was applied on *D. abyssinica* at 14 days after sprouting (4^{th} or 5^{th} node growth stage) and cotton at 16 days after germination (4^{th} - 6^{th} leaf stage). The leaves of both plant species were excised and collected in polythene bags at intervals of 2, 8, and 48 h after the herbicide application. The excised leaves were stored in a freezer (-80^{0}C).

5.2.1. Procedure for plant tissue extraction

Plant tissues (frozen leaves) were weighed and cut into small pieces, prior to homogenising using an Ultra Turrax T 25 homogeniser (2x10 seconds at 15000 rpm). A 1:5 w/v (wt of plant tissue volume of extraction buffer) extract was prepared for analysis of neutral (cytoplasmic) proteases, the extraction buffer for which consisted of 50mM Tris-acetate buffer pH 7.5, 1mM dithiothreitol (DTT), 0.15M NaCl and 3mM Na azide, while the acidic (vacuolar) proteases extraction buffer was as above but contained 50mM acetate buffer pH 5.5. Homogenates were centrifuged at 2000 rpm for 7 minutes at 6^{0}C and the supernatants retained for protease assays.

5.2.2. Assays of proteases

0.05 ml supernatant was incubated with the appropriate assay medium (total volume 0.3 ml) at 37^{0}C for 10-120 minutes, and the reaction terminated by addition of 0.6 ml of ethanol. The fluorescence of the liberated aminoacyl 7-amino-4-methylcoumarin (AMC) was measured by reference to a tetraphenylbutadiene fluorescence standard block ((ex 370 nm, (em 430 nm). Assay blanks were run with assay medium minus enzyme

samples. The stock substrate solutions (2.5 mM) were prepared in 10% ethanol. The various proteases assayed, were selected to represent various intracellular locations and substrate specificities as follows;

Neutral proteases

Alanyl Aminopeptidase: 50mM Tris-acetate buffer pH 7.5, 5mM $CaCl_2$, 1mM DTT, 0.25mM Ala-AMC;

Arginyl Aminopeptidase: 50 mM phosphate buffer pH 6.5, 0.15M NaCl, 1 mM DTT, 0.25 mM Arg-AMC;

Dipeptidyl Aminopeptidase 1V: 50 mM Tris-acetate buffer pH 7.5, 1 mM DTT, 0.25 mM Gly-Pro-AMC;

Tripeptidyl Aminopeptidase : 50 mM Tris-acetate buffer pH 7.5, 2 mM DTT, 0.25 mM Ala-Ala-Phe-AMC;

Acid proteases

Dipeptidyl Aminopeptidase 1: 50 mM acetate buffer 5.5, 2 mM DTT, 0.25 mM Gly-Arg-AMC;

Dipeptidyl Aminopeptidase 11: 50 mM acetate buffer pH 5.5, 2 mM DTT, 0.25 mM Lys-Ala-AMC;

Cathepsin B + L: 50 mM acetate buffer pH 5.5, 2 mM DTT, 0.25 mM CBZ-phe-arg-AMC;

Cathepsin B: 50 mM acetate buffer, pH 5.5, 2 mM DTT, 0.25 mM CBZ-arg-arg-AMC;

5.2.3. Determination of soluble protein

The levels of soluble proteins in the supernatants used for the assays of the protease types investigated in the study, were determined as described by (Bradford, 1976). Bradford reagent was prepared using coomassie brilliant blue G-250 (100 mg), which was dissolved in 95% ethanol (50ml), thereafter orthophosporic acid (100 ml of 85% (w/v) was added. The solution was diluted with deionised water to a one litre. The protein solution (0.1 ml) was pipetted in, to a test tube where 5 ml of the Bradford reagent was added. The contents were placed on a shaker for a few seconds for thorough mixing. Then the absorbance was measured at 595 nm on a spectrophotometer against a blank in which the experimental buffer (Tris-HCL, pH 8.0) was placed instead of the volume of protein solution. The absorbance values were taken corresponding with the known concentration of the protein solution. Then the soluble protein content was calculated;

$$\text{Protein content (mg/g)} = \frac{\text{Specific activity (nmol/ml/h)}}{\text{Protein conc. in the mixture}}$$

Figure 5.1. represents the standard curve for determination of the unknown protein content of *D. abyssinica* and cotton homogenates.

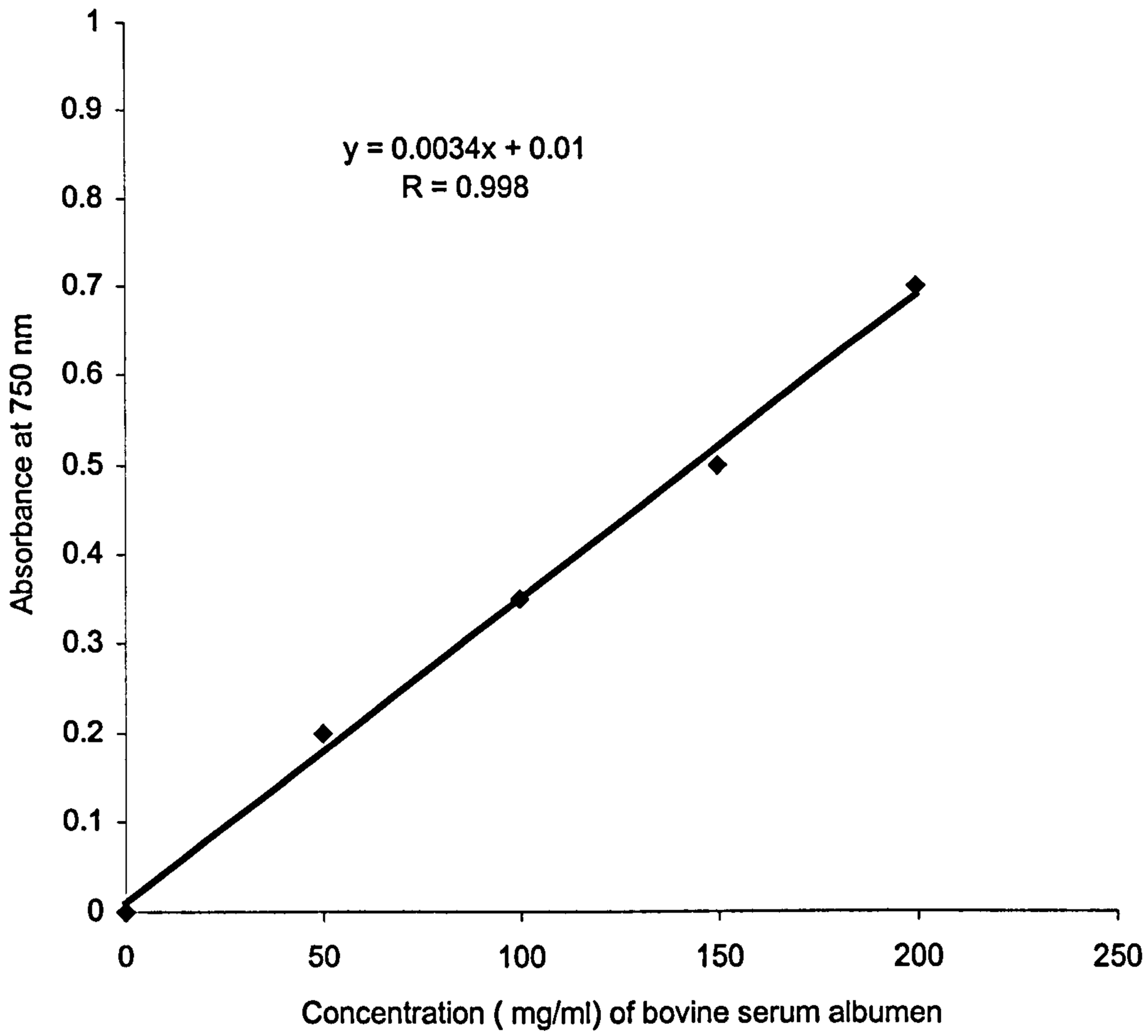


Figure 5.1. Standard curve to determine soluble protein concentration of *D. abyssinica* and cotton homoganates.

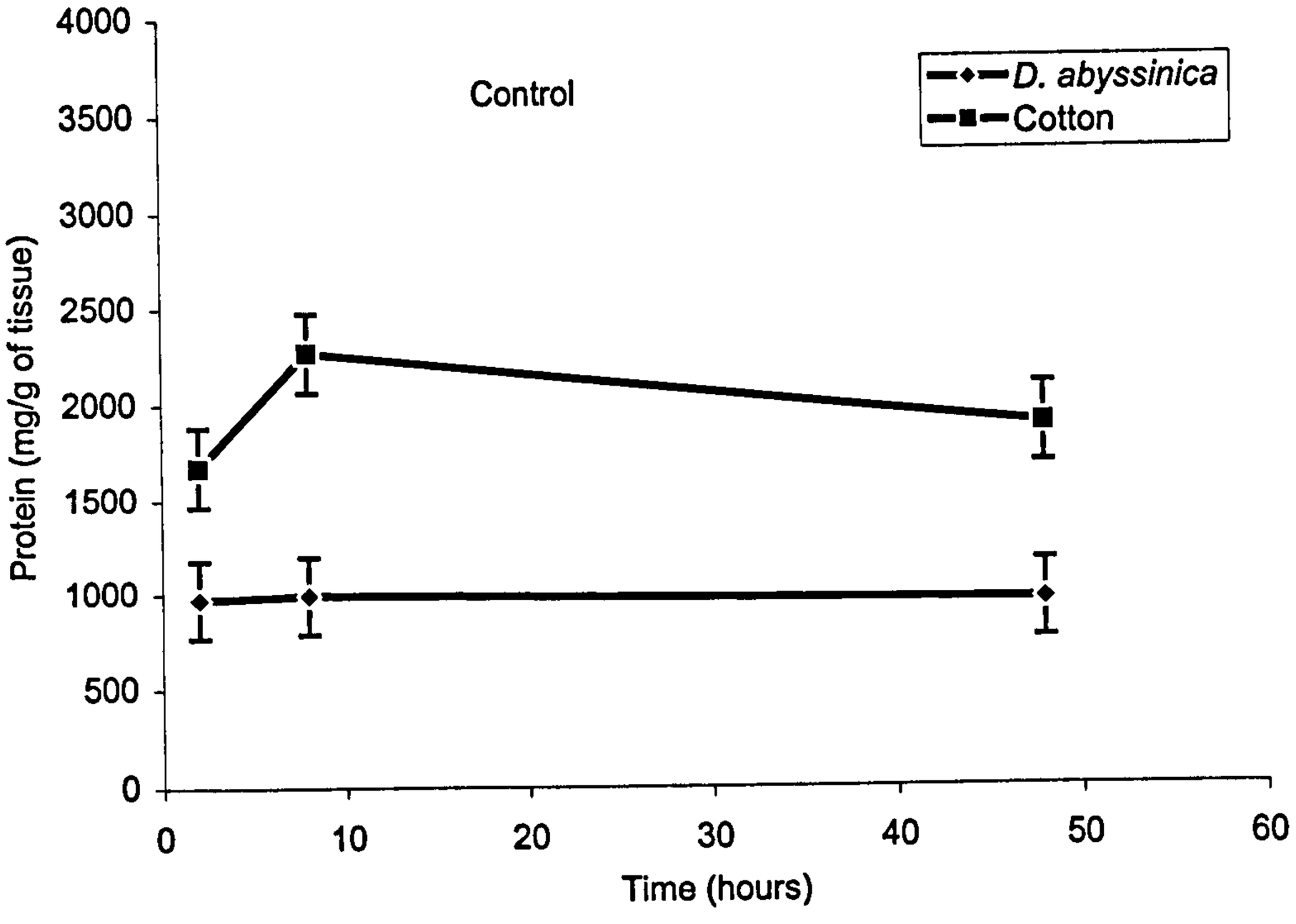
5.2.4. Data analysis

The data recorded on the soluble protein content and protease activities of *D. abyssinica* and cotton plants was subjected to analysis of variance (ANOVA). The means were separated at 5% level significance using Tukey's multiple rage test.

5.3. Results and discussion

5.3.1. Soluble protein content in the plant tissue extracts of the treated and untreated *D. abyssinica* and cotton (leaves).

It has been reported that abnormal proteins in plants continually arise by a variety of mechanisms which can be accelerated by environmental stress (Gatenby and Vitanen, 1994; Maurizi, 1992; Viestra, 1993). However, for some proteins or in situations where the levels of abnormal proteins become too high, proteolysis is an important solution (Gatenby and Vitanen, 1994). In this study protein content was measured in *D. abyssinica* and cotton plants after the application of sethoxydim to determine its effect on the proteins of the two plant species. Figure 5.2 illustrates the soluble protein content obtained from plant tissue extracts of *D. abyssinica* and cotton. The protein content was measured at times in both plant species after treatment with sethoxydim. It was found that the soluble protein content was significantly high in cotton compared to *D. abyssinica* irrespective of whether the plants were treated or not. In *D. abyssinica* constant protein content at all times was observed in the control whereas in cotton it was noted highest at h 8. Results showed that the application of sethoxydim resulted in significant changes on



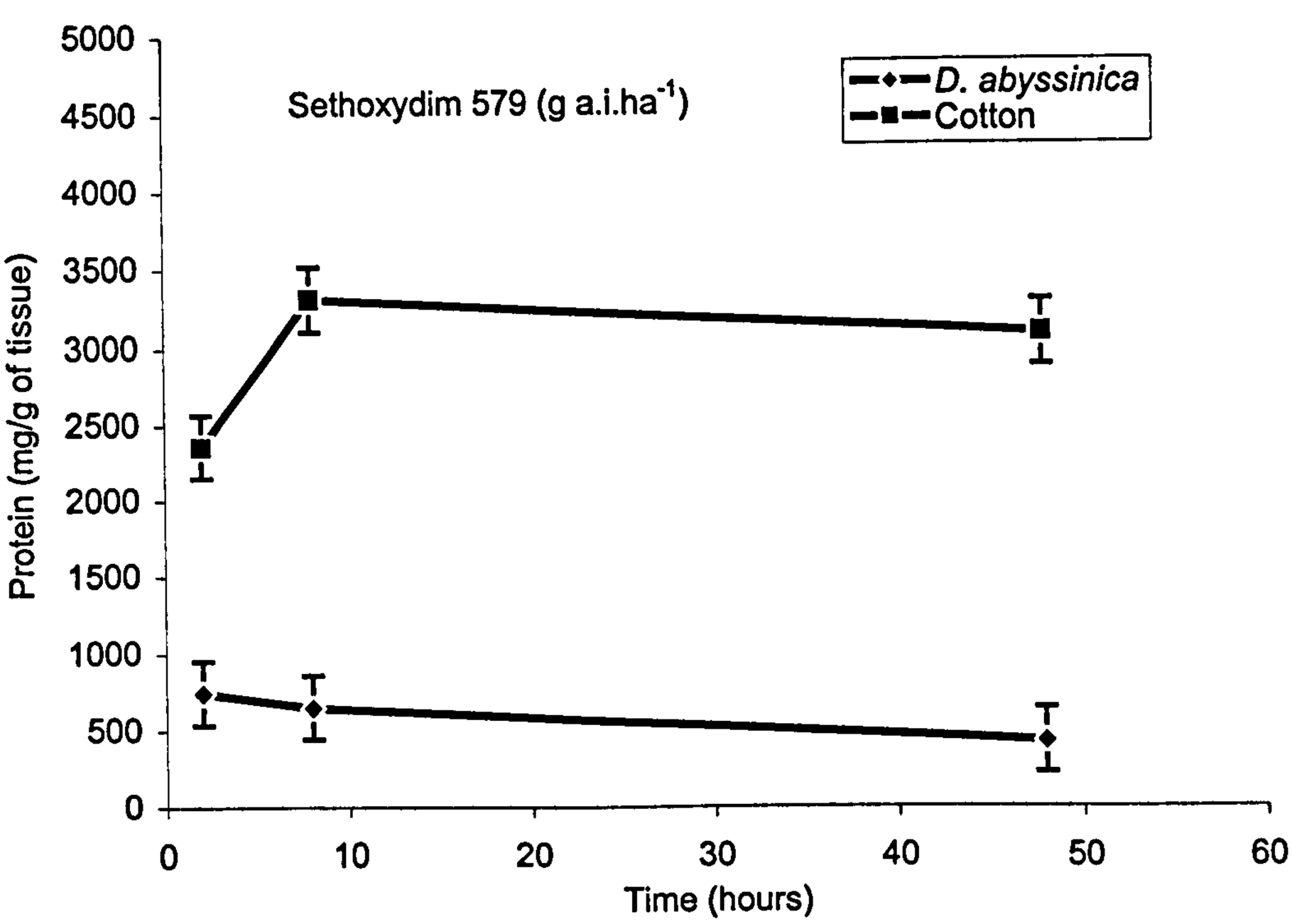


Figure 5.2.Total soluble protein content in susceptible *D. abyssinica* and tolerant cotton plants obtained at times after herbicide application in the greenhouse.
Bars represent standard error of each mean value of two replications.

the soluble protein content in *D. abyssinica* (Appendix 5.1). Significantly less protein content was noted at all times after herbicide application compared to the control. It can therefore be assumed that sethoxydim interfered with protein hydrolysis *in vivo* or it possibly reduced the activity of enzymes responsible for the protein hydrolysis in *D. abyssinica*. Moreland *et al.*, 1969; Grzesiuk *et al.*, 1971; Tonecki, 1975a, have also reported decrease in protein content in plants due to herbicides such as dalapon and 2,4,5-T. Protein content in cotton was however, less affected by sethoxydim. Results revealed that there was significant increase in proteins after treatment especially at h 48 compared to the control, indicating that protein synthesis in cotton was not negatively affected by the herbicide.

5.3.2. Proteases activity in the untreated *D. abyssinica* and cotton plant tissue extracts (leaves).

The assays for intracellular proteases in this study were based on corresponding enzymes originally investigated in higher animals, using specific fluorometric methods to determine the activities of individual enzymes (Faiz *et al.*, 1994). These activity levels of the protease investigated in higher animals were also found similar in plants (O'Cuinn, 1998). Generally, classification of proteases is based on the pH optimum activity (acidic, neutral or alkaline), size of substrate (proteins and peptides) and nature of enzyme active site (serine, cysteine, aspartic, metallo). Comparison of intracellular protease activities in the untreated *D. abyssinica* at 14 days after sprouting and untreated cotton at the 6th leaf stage (16 days after germination) is illustrated in Figures 5.3. Results obtained showed

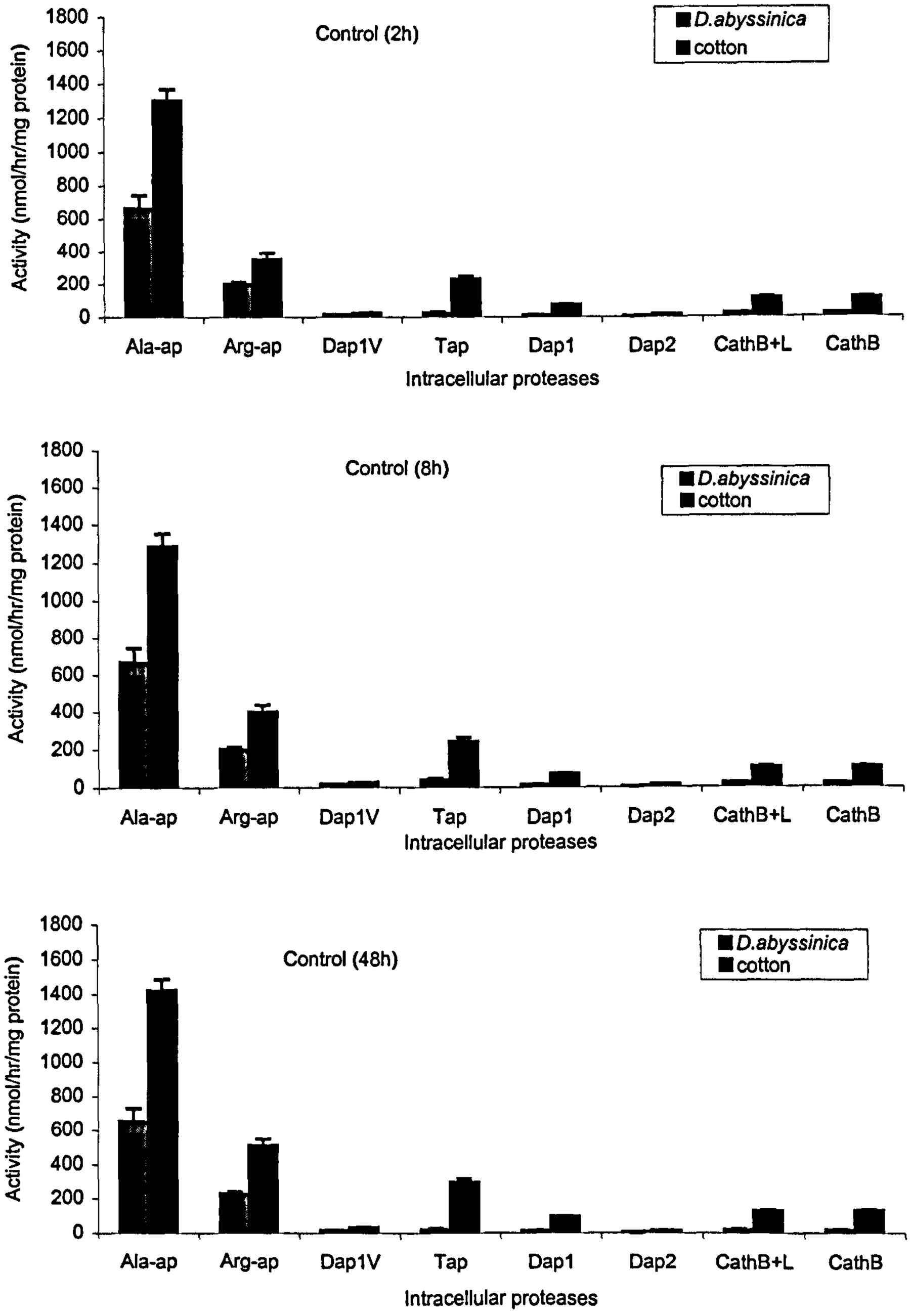


Figure 5.3. Activity levels of the intracellular protease in the untreated plants of cotton and *D. abyssinica* measured at times in the greenhouse. Bars represent standard error of each mean value of two replications.

that the activity levels of both neutral and acidic protease were significantly higher in cotton than in *D. abyssinica* in the at times (Appendices 5.1, 5.2, 5.3, 5.4, 5.6, 5.7, and 5.8). In both cotton and *D.abyssinica* plants, the activity level of alanyl aminopeptidase was noted highest compared to the activity levels of other proteases assayed in untreated plants, but it was significantly high in cotton compared to *D. abyssinica*. This was noted at all times. The activity of arginyl aminopeptidase was also relatively high in the untreated plants of cotton and *D. abyssinica* but it was significantly less in *D. abyssinica* compared to cotton. Activities of other proteases such as tripeptidyl aminopeptidase, dipeptidyl aminopeptidase, cathepsin B+L and cathepsin B were also significantly high in cotton compared to *D. abyssinica*. In general terms, activity levels of the intracellular proteases assayed are significantly high in cotton compared to those observed in *D. abyssinica*. This is also reflected in the soluble protein content of the two plant species, since proteases are part of the proteins, and play a key role in their degradation.

5.3.3. Proteases activity in treated plant tissue extracts of cotton and *D. abyssinica* (leaves).

It has already been mentioned in this study that the application of sethoxydim to *D. abyssinica* and cotton plants, stressed *D. abyssinica* but cotton was tolerant to the herbicide. This therefore initiated the investigation on the role of protease activities of the two plant species with emphasis on their response to the herbicide. According to Wilkins *et al.*, (1999), the activity levels of neutral and acidic proteases were noted significantly high in resistant biotypes compared to susceptible ones (personal communication).

Research elsewhere indicated increase of protease activity in castor bean and maize plants after the application of metribuzin (Elsaht *et al.*, 1994), while the protease activity levels in sweet corn were correlated with the application of atrazine (Khodary, 1990). However, the effects of herbicides on protease activities in plants have not yet been extensively documented. Results obtained in this study indicated significant changes in the activity levels of some of the intracellular protease in the treated *D. abyssinica* and cotton plants at times (Figure 5.4). In *D. abyssinica*, a significant decrease in the activity levels of arginyl aminopeptidase (arginyl-ap) and tripeptidyl aminopeptidase (Tap) was noted at hours 8 and 48. While in the treated cotton tripeptidyl aminopeptidase significantly increased at times. However, further observation found that there were no significant changes in the activity levels with the interaction of time, treatment and species (Appendices 5.2,5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9).

5.3.4. Activity levels of the intracellular protease in the treated and untreated cotton plant tissue extracts (leaves).

Observation done on the treated and untreated cotton plants showed that the activity levels of both neutral and acidic proteases were less affected by the sethoxydim, but the herbicide significantly induced the increase of the activity level of tripeptidyl aminopeptidase in the treated cotton plants at hour 2 and 48 (Figure 5.5 and Appendix 5.5). This increase of the proteolytic activity in cotton may be the advantage for this crop to be tolerant by increasing the supply of free amino acids to the intracellular pool, either *de novo* synthesis of known herbicide metabolising enzymes such as cytochrome P450,

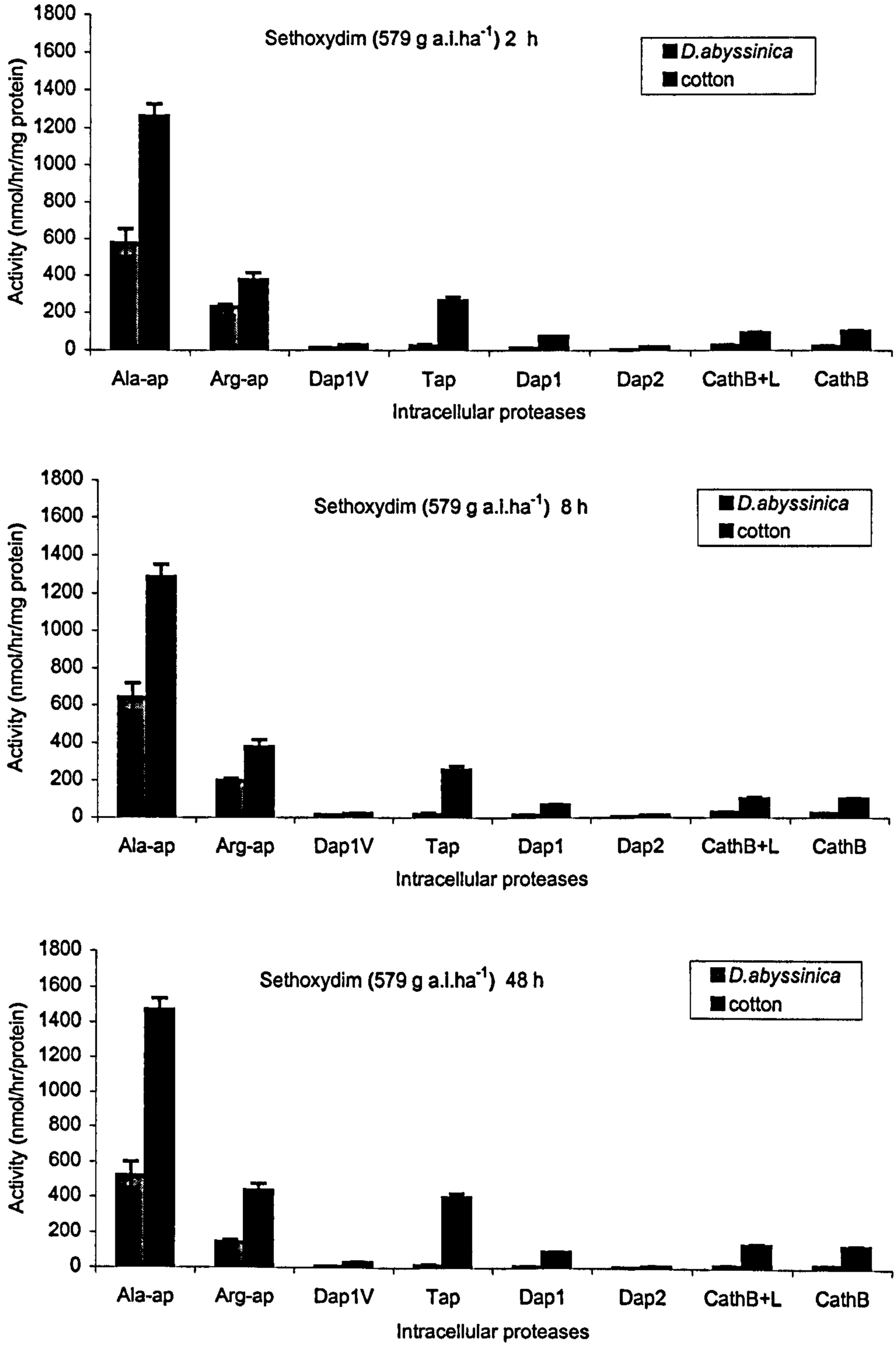


Figure 5.4. Activity levels of the intracellular protease in the treated plants of *D. abyssinica* and cotton measured at times in the greenhouse. Bars represent standard error of each mean value of two replications.

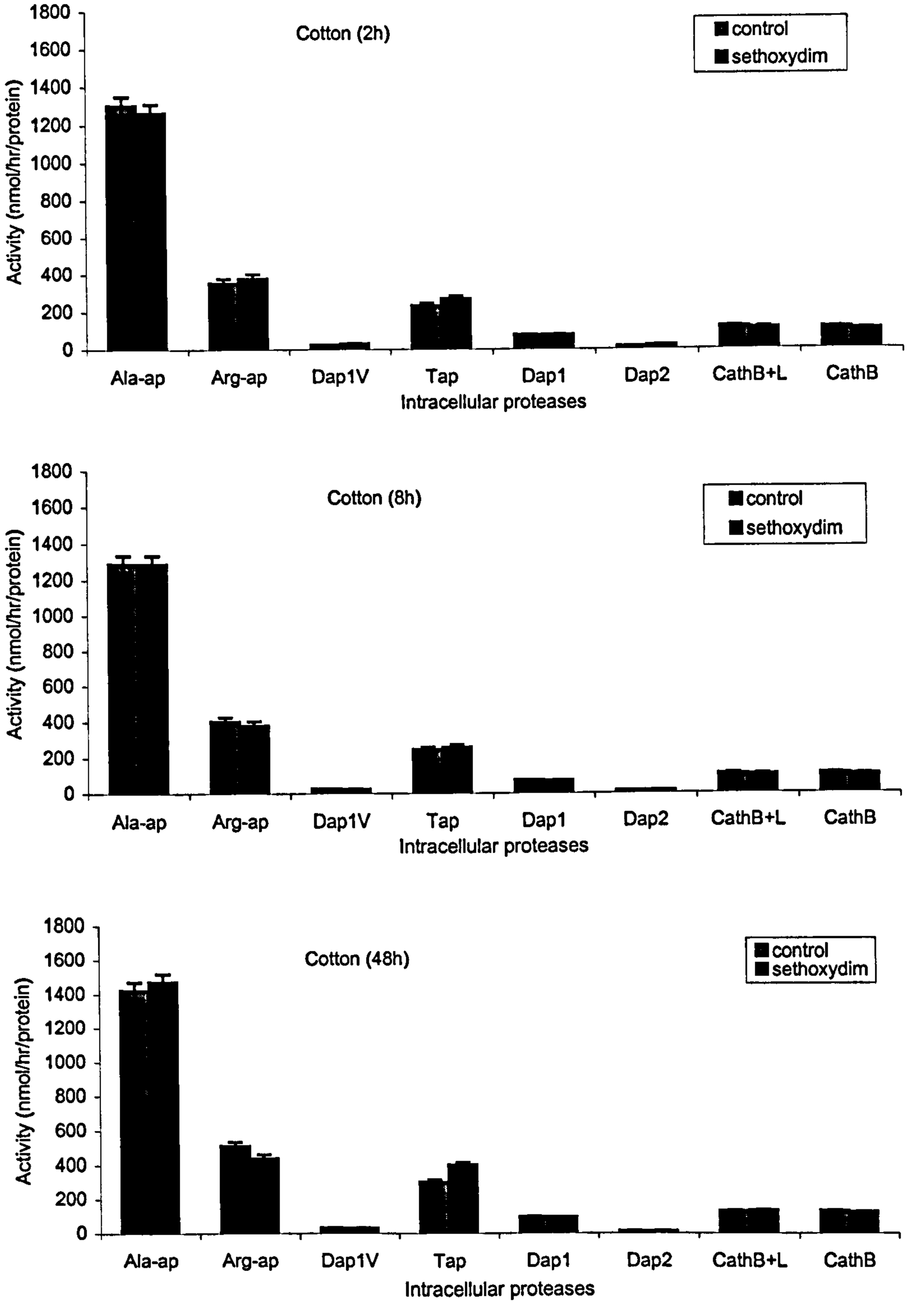


Figure 5.5.Activity levels of the intracellular protease in the treated and untreated plants of cotton measured at times in the greenhouse. Bars represent standard error of each mean value of two replications.

or increased biosynthesis of secondary plant substances such as phenolics, alkaloids, glucosinolates or glycoside antifeedants derived from amino acid precursors (Liu *et al.*, 1994). According to Walling *et al.*, 1995), the wounding of tomato or potato plants induce defence related proteins/enzymes such as leucine aminopeptidase, aspartic proteases and cysteine protease inhibitors, in addition to polyphenol oxidases. As it is known that protease activities broadly increase to a similar degree for both herbicide and insect resistant plants, this phenomenon might be part of the plant defence mechanism to stress, in this case cysteine proteases were reported to have accumulated in the plant tissues exposed to environmental stress (drought) (Koizumi *et al.*, 1993).

5.3.5. Activity levels of the intracellular protease in the treated and untreated *D. abyssinica* plant tissue extracts (leaves).

In a comparison of the treated and untreated *D. abyssinica*, a significant decrease in the activities of arginyl aminopeptidase at h 48 and tripeptidyl aminopeptidase at h 8 were observed compared to other proteases assayed (Figure 5.6 and Appenidices 5.3 and 5.5). This significant decrease in the activity levels of these proteases was a result of the application of sethoxydim, this observation was different from what was noted in treated cotton plants, especially for tripeptidyl aminopeptidase. It can therefore be assumed that the susceptibility of this weed might partly be due to the significant reduction of some of these intracellular proteases. Herbicides such as thiocarb and butachlor have also been reported to have caused decrease of protease activity in *Enchinochloa crus-galli* (Kumar and Prakash, 1994). It is also possible that the significant decrease of soluble proteins in

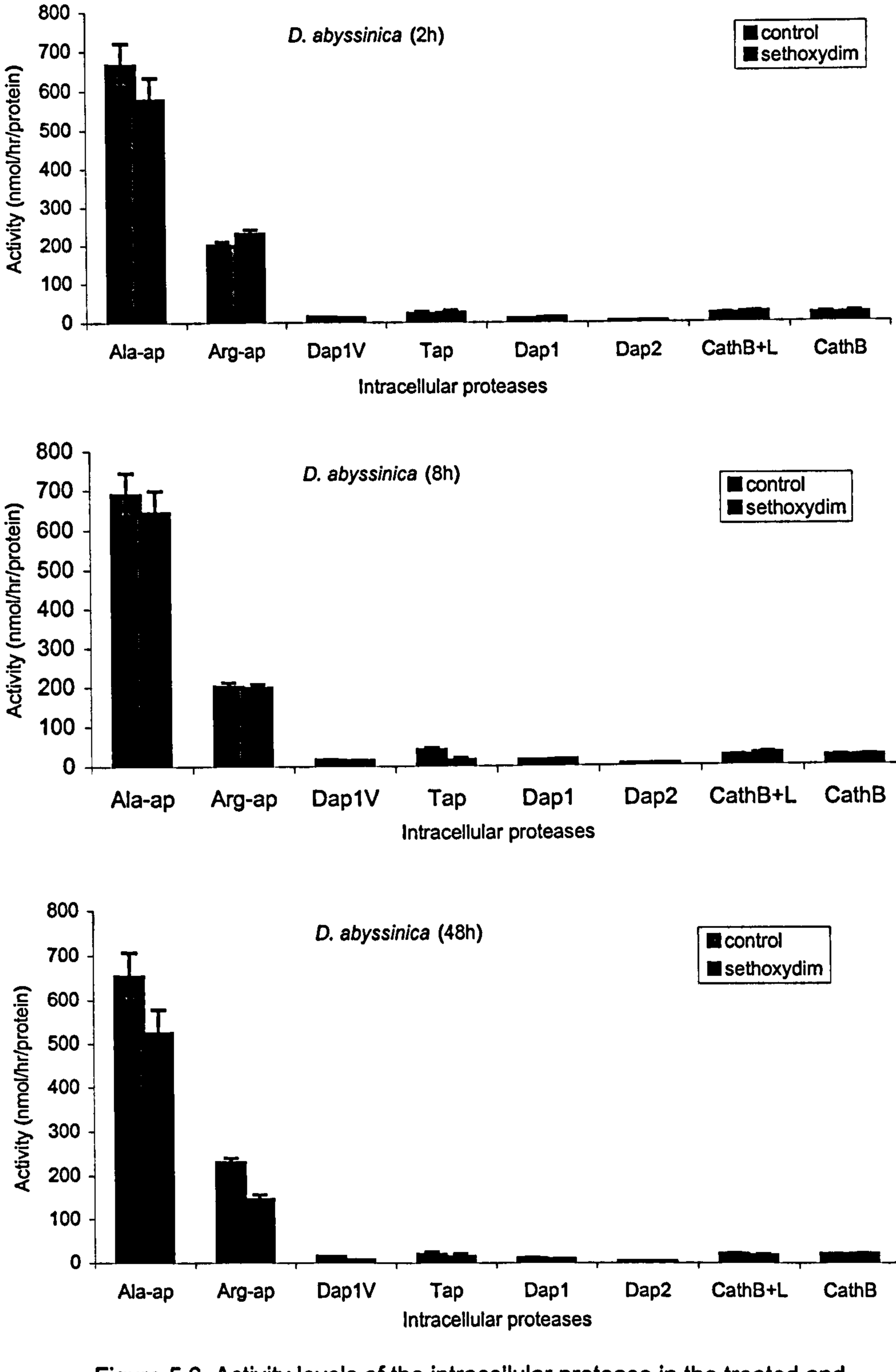


Figure 5.6. Activity levels of the intracellular protease in the treated and untreated plants of *D. abyssinica* measured at times in the greenhouse.
Bars represent standard error of each mean value of two replications.

D .abyssinica after treatment might have been due to the decrease in the activity levels of some of these intracellular protease, as it was noted in section 5.3.1. As reported by Vierstra, (1996), protein degradation is an essential component in most aspects of plant growth, development and environmental responses. On the other hand, the tolerance of cotton to sethoxydim unlike *D. abyssinica* is because the herbicide is a grass selective, thus broadleaf weeds/crops are tolerant to the herbicide. In general terms, weed or crop tolerance/resistance to a herbicide may be due to a detoxification mechanism reducing the amount of herbicide to reach the site of action (Clay, 1989). In susceptible plants, sethoxydim inhibits acetyl CoA carboxylase while in broadleaf plants the enzyme is insensitive to the herbicide (Burton *et al.*, 1987 and Harwood, 1991). One of the possibilities that a weed/crop becomes resistant to the herbicide is an overproduction of the target site of enzyme in the plants. Studies conducted by Gronwald *et al.*, (1989) indicated over production of acetyl coA carboxylase as a possible mechanism of maize cell cultures resistance to sethoxydim and haloxyfop. However, according to Devine *et al.*, 1993, herbicides usually have multiple interactions at various levels within the plant. These interactions begin immediately when the herbicides enter the plant, and this is quickly followed by a series of steps that precedes the arrival of the herbicides at their site of action. It can therefore be assumed that the inhibition of the activity levels of some of the intracellular protease was one of the interactions of sethoxydim within *D. abyssinica* plants.

CHAPTER SIX

GENERAL DISCUSSION

6.1. The main purpose of the study

The major concern of the study was to determine the appropriate dose rate(s) of sethoxydim and fluazifop-butyl for adequate control of *Digitaria abyssinica* in cotton production in Uganda. This was specifically done by evaluating, 1) the efficacy of the reduced dose rates of sethoxydim and fluazifop-butyl supplemented with two hand-hoe weedings and evaluate their economic benefits, 2) investigating the responses of *D. abyssinica* to the dose rates tested.

6.2. Percentage weed control of *D. abyssinica* with reduced dose rates of fluazifop-butyl and sethoxydim.

D. abyssinica is a highly competitive grass weed species (Popay and Ivens, 1982). Results obtained from this study showed that sethoxydim and fluazifop-butyl adequately controlled *D. abyssinica*. The control of this weed gave favourable conditions for the growth of high densities of broadleaf weeds such as *Oxalis latifolia* (Figure 2.2). These broadleaf weeds were not difficult to control with the hand weeding supplements. The overall mean percentage control of *D. abyssinica* after the application of fluazifop-butyl and sethoxydim was significantly high compared to the control. Data obtained from the field experiments in the two seasons showed that the reduced dose rates of sethoxydim

(405, 502, g a.i.ha^{-1}) and fluazifop-butyl (138, 162, g a.i.ha^{-1}) provided almost complete control of *D. abyssinica* at the two sites. At the Namulonge site weed control levels of 79-92% were obtained for fluazifop-butyl and levels of 91-96% for sethoxydim. A similar trend was obtained at Bukalasa, where weed control percentages ranged between 81-91% for fluazifop-butyl and 90-94% for sethoxydim. The high activity of sethoxydim against *D. abyssinica* was reported by Parker, (1982). The activity of fluazifop-butyl and sethoxydim against other grass weed species has also been reported by Moreland, (1980) and Peters, (1980). The present study confirmed that the reduced dose rates did not significantly differ in the percentage control of *D. abyssinica*. Generally, it was noted that there was no dose response relationship for both herbicides at the two sites in the two seasons for the range of dose rates tested, indicating the potential activity of the reduced dose rates against *D. abyssinica*. Therefore this study has demonstrated that herbicide doses below the recommended rates selectively and adequately controlled *D. abyssinica*.

6.3. Reduction of *D. abyssinica* shoots and rhizomes with fluazifop-butyl and sethoxydim.

Sethoxydim and fluazifop-butyl were applied to the foliage of *D. abyssinica*. These herbicides caused chlorosis and necrosis on the leaves of *D. abyssinica* within 5-14 days after application which was an early indication of plant phytotoxicity. According to Carr *et al.*, 1986, grass weed species treated with fluazifop-butyl and sethoxydim usually show such symptoms as an indication of growth inhibition of the treated plants when the meristems cease to function and as a result the young leaves appear necrotic.

Following the chlorosis and necrosis of *D. abyssinica*, the fresh and dry weights of shoots and rhizomes of the weed were assessed both in the field and greenhouse. Substantial reduction of fresh and dry weights of *D. abyssinica* shoots and rhizomes followed the application of fluazifop-butyl and sethoxydim at reduced and full dose rates. These results agreed with the research findings obtained by Chandrasena and Sagar, (1984); Kells *et al.*, (1984); Swisher and Corbin, (1982) when they studied the effect of fluazifop-butyl and sethoxydim at reduced and recommended rates on various grass weed species. Analysis of variance showed that the reduction of fresh and dry weights of *D. abyssinica* shoots and rhizomes did not significantly differ amongst dose rates. On average fresh weights were reduced by 30-60% and dry weight by 50-64% for both shoots and rhizomes. The lowest dose rates (below half of the full rates), sethoxydim (116 g a.i.ha^{-1}) and fluazifop-butyl (38 g a.i.ha^{-1}) gave the lowest percentage reduction compared to the other dose rates. In another observation, it was noted that there was a high correlation between the percentage loss of water in *D. abyssinica* and the herbicide dose rates. This relationship indicated further damage of the herbicides to the *D. abyssinica* plants.

6.4. Plant stress due to fluazifop-butyl and sethoxydim applications

Investigation of responses of *D. abyssinica* to the individual dose rates of sethoxydim and fluazifop-butyl was another objective in this study. Therefore plant stress of the weed was measured through various ways.

6.4.1. Chlorophyll content

Chlorophyll content of *D. abyssinica* was measured at the end of the experiment in the greenhouse, 29 days after herbicide application because the chlorophyll meter was not available during the experimentation period. The general chlorosis which appeared on the *D. abyssinica* leaves following the application of sethoxydim and fluazifop-butyl gave indications of loss of chlorophyll content as part of the death process of the plants. Most plant bleaching herbicides are associated with yellow chlorosis of the leaves which is a result of total or partial absence of normal chloroplast pigments such as chlorophyll (Britton *et al.*, 1989). Therefore chlorophyll content has been among the parameters evaluated for the herbicidal activity against weeds. Results obtained in the present study indicated that the application of fluazifop-butyl and sethoxydim on *D. abyssinica* decreased chlorophyll content (40-70%) in the plants while the chlorophyll content in the controls remained fairly constant. All dose rates of both herbicides had a significant effect on the chlorophyll content compared to the control. This is an indication that fluazifop-butyl and sethoxydim influenced the decrease of chlorophyll content in the treated plants of *D. abyssinica*. The results supported research findings reported by Fletcher and Kirkwood, (1982) for other grass species which were also treated with sethoxydim and fluazifop-butyl. On the other hand, Kitchen *et al.*, (1981) suggested that the reduction of chlorophyll content following herbicide application may be a result of chlorophyll-synthesis inhibition or increased chlorophyll degradation. In another study, herbicides such as glyphosate were reported to affect chlorophyll content through photobleaching (Canal Villanueva *et al.*, 1985).

Although analysis of variance showed no significant differences amongst dose rates of sethoxydim and fluazifop-butyl in relation to reduction of chlorophyll content, regression analysis seemed to suggest that the loss of chlorophyll content was significantly associated with the herbicide concentrations. This is mainly demonstrated with the fluazifop-butyl dose rates.

6.4.2. Measurements of fluorescence

Photosynthetic efficiency of most plants is decreased when they are exposed to stress. To date fluorescence measurements has been used as one of the techniques to assess plant stress. According to Devine *et al.*, 1993, fluorescence measurement is used to quantify *in vivo* herbicide interference with photosynthetic electron transport from dark-adapted leaf illuminated with a flash of light. In the present study fluorescence was measured from the *D. abyssinica* leaves after the application of sethoxydim and fluazifop-butyl under both field and greenhouse conditions. The minimal fluorescence (Fo) and maximum fluorescence (Fm) were altered in the treated *D. abyssinica* plants compared to the untreated. The values of these parameters were highly increased in the treated plants, possibly indicating plant injury due to the herbicides. Arntzen *et al.*, 1982; Devine *et al.*, 1993 reported that Fo and Fm increase in treated plants if the transfer of electrons to Q_B (secondary electron acceptor) and into the plastoquinone is inhibited by a herbicide. Herbicides such as diuron have been observed to stimulate fluorescence through inhibition of Q_A (primary electron acceptor) reoxidation (Duysens and Sweers, 1963).

It has also been noted that the mechanism of herbicide action is a displacement of Q_B from its binding site at the Q_B-protein (Velthuys, 1981). In addition, most phenoxyacetic acids inhibit the photosynthetic electron transport by binding to the photosystem II reaction centre protein D-1 at the binding site of the plastoquinone (Barber, 1987; Trebst, 1986; Mattoo *et al.*, 1989). In this study however, there is a possibility that sethoxydim and fluazifop-butyl could have affected the reaction centre in PSII blocking the flow of electrons which led to increasing Fo and Fm in the treated *D. abyssinica*. Measurement of Fv/Fm in *D. abyssinica* leaves drastically decreased in the treated plants compared to the untreated. This further confirmed the damage of the plants due to herbicide application, especially in cases where the increase of Fo and Fm was associated with the reduction of Fv/Fm. Most of the herbicide dose rates tested reduced Fv/Fm with an average reduction of 0.36-0.56 compared to the control 0.73-0.78. These results indicated overall reduction in the efficiency of photochemistry of *D. abyssinica* and as a result the plants died. According to Björkman and Demmig, (1987) Fv/Fm provides information on the efficiency of photochemistry of photosystem II. On the other hand, fluorescence measurements can give an estimation as to when the herbicides started being active against the weed. In the case of *D. abyssinica*, plant injury due to fluazifop-butyl and sethoxydim seemed to have started a few hours after the application of the herbicides and it was pronounced at 5-14 days after treatment. In this study, fluorescence parameters were not recorded from cotton plants because the crop is tolerant to sethoxydim and fluazifop-butyl and there were no stress symptoms observed.

6.4.3. Activity of intracellular proteases in *D. abyssinica* and cotton plants in response to sethoxydim.

Proteases play a key role in the physiological processes that are important to agriculture such as storage protein breakdown, development which includes senescence and intracellular turnover. Most proteases appear to act together to breakdown proteins to produce peptides and amino acids which can be reutilized by cells or transported to other plant tissues. According to Clarke, (1999) proteases are recognised for performing vital functions throughout the cell. Investigation done on the soluble protein content of the treated and untreated *D. abyssinica* and cotton plants gave an indication that cotton had significantly high proteins compared to *D. abyssinica*, possibly suggesting high proteolytic activity in cotton. The soluble protein content was significantly reduced in the treated *D. abyssinica* unlike in the treated cotton, where the proteins significantly increased. This might be one of the possibilities why *D. abyssinica* was stressed. As it was reported by People and Dalling, (1988); Huffaker, (1990); Feller and Fischer, (1994) that the loss of protein is a dominant feature of leaf senescence. The assay of the intracellular proteases also revealed that the enzymatic activities were high in cotton compared to *D. abyssinica*, in both treated and untreated plants of the two species. However, both *D. abyssinica* and cotton plants showed a high constitutive level of intracellular protease alanyl aminopeptidase at times irrespective of whether the plants were treated or untreated, giving an implication that this enzyme might not have any role to play in the susceptibility of the weed or tolerance of cotton to sethoxydim. The activity levels of arginyl aminopeptidase and tripeptidyl aminopeptidase were also relatively high

in the untreated *D. abyssinica*, but they drastically decreased with time after treatment. A significant decrease in the activity levels of arginyl aminopeptidase (36.1%) and tripeptidyl aminopeptidase (51.8%) was noted in the treated plants of *D. abyssinica* at h 48 and 8 respectively after herbicide application, suggesting their possible role in the susceptibility of the weed. Other proteases assayed, had fluctuating activity levels at times in both treated and untreated plants of *D. abyssinica*. According Grzesiuk *et al.*, 1971; Maštakov *et al.*, 1971; Merezhinskii *et al.*, 1972 and Ploszy ski, 1972, changes in enzymatic activity due to herbicides indicates an association of active substances of the herbicides with the enzymes. While Grzesiuk and Sójka, (1970) and Ploszynski, 1972 reported that these enzyme activities may change after herbicide application due to changes in level of natural growth regulators which also regulate the enzymatic activity. In another study however, Merezhinskii *et al.*, 1971; Moreland *et al.*, 1969 and Ploszy skii, 1972 noted that the changes in the activity of enzymes following the application of herbicides could be due to inhibition of synthesis of enzymes *de novo*. On the other hand, the activity levels of some of the intracellular proteases in the treated cotton plants did not change, while others significantly changed with time. For example the activity level of tripeptidyl aminopeptidase significantly increased at h 48 in the treated cotton plants. The increase in the protease activity in the treated cotton plants might have been a mechanism to synthesis more enzymes to overcome the herbicidal stress. An increase of protease activity to overcome herbicidal stress has been reported by Kumar and Prakash, (1994) when they applied thiobencarb and butachlor on barnyard grass (*Echinochloa crus-galli*).

6.5. Influence of weed control on the crop performance

Crop yield losses due to high weed infestation has been a world-wide concern. Cotton like other crops is affected by weed competition in the field and yet it is one of the crops which are difficult to grow due to its high labour demand and input requirements. The combination of reduced and full dose rates of sethoxydim and fluazifop-butyl with 2 hand weeding supplements was investigated in the study for the control of perennial, annual grasses and broadleaf weeds in cotton. Cotton was not injured by the herbicides, if any injuries, they were negligible or possibly the crop was tolerant. This is reflected in the data recorded on the growth and development of the cotton plants from the treated and untreated plots. All the treatments used suppressed *D. abyssinica* and other weeds in cotton, although the percentage weed control of *D. abyssinica* and other weed species was not obtained from the hand weeding (5 times) treatment. However, the combinations of herbicides and 2 hand weeding supplements gave as good as or better cotton crop as the one obtained from the hand weeded plots. This was noted at Namulonge and Bukalasa during the two seasons, despite the poor season of 1997/98. Results suggested that in the 1995/96 season the cotton crop yielded highly at Namulonge compared to Bukalasa. This could have resulted from the soil fertility variations of the two sites (Table 2.2). The cotton yields obtained during the poor season 1997/98 were not quite promising and as a result the cotton crop at the two sites yielded equally poor. On average, the cotton yields ranged between 1793-2993.7 kg ha^{-1} from the combinations of herbicides and hand weeding for Namulonge and Bukalasa in 1995/96 compared to 2400-2681.2 kg ha^{-1} from the weeded plots. While in 1997/98 season the combinations gave yields of

665-1184.2 kg ha^{-1} for Namulonge and Bukalasa compared to 898-919 kg ha^{-1} from the weeded plots. When cotton plants were left under heavy weed infestation, reduction of plant height and branches was 30-44% and 60-70% respectively, and the seedcotton yields were reduced by more than 80%. These results agreed with the research findings reported by Vencill *et al*, (1992) who assessed cotton yield losses when bermudagrass (*Cynodon dactylon*) was allowed to compete with cotton. Results obtained from the economic analysis gave the possibilities of how the combination of reduced dose rates and 2 supplements of hand weeding can be useful and practical in weed management in cotton for a smallholder cotton farmer. Although however, not all treatments used in the study were of a convincing advantage for the small farmer.

6.6. Summary and Conclusion

The broad objective to determine the appropriate dose rate(s) to control *D. abyssinica* in cotton production in Uganda was studied. A number of herbicides have been used to control *D. abyssinica* in various crops. However, the use of fluazifop-butyl and seythoxydim for the control of *D. abyssinica* in cotton production has not yet been extensively documented. This study has confirmed that the reduced dose rates of these herbicides adequately controlled *D. abyssinica* both in the field and greenhouse. However, the lowest dose rates (below half of the full rates) indicated a short time control of this weed. Cotton plant phytotoxicity resulting from the applications of the fluazifop-butyl and sethoxydim was not observed. Therefore the dose rates of fluazifop-butyl at 94, 138, and 162 g a.i.ha^{-1} and sethoxydim at 290, 405 and 502 g a.i.ha^{-1} were found

appropriate and as good as the full rates (fluazifop-butyl 188 g a.i.ha^{-1} and sethoxydim 579 g a.ha^{-1}) in controlling *D. abyssinica*. This is confirmed from the results obtained on most parameters measured on *D. abyssinica*. The results have also confirmed that application of sethoxydim and fluazifop-butyl inhibited plant growth, chlorophyll content and interfered with the photosynthetic electron transport in the PSII of *D. abyssinica* leaves. The activities of some of the intracellular proteases were also negatively affected following the application of sethoxydim on *D. abyssinica*, unlike in cotton where some of these proteases were positively affected following the application of the herbicide. There is a possibility that the differences in the protease activity of these two plants species might be associated with their response to sethoxydim. Generally, the results have showed that reduction of chlorophyll content, inhibition of photosynthetic electron flow and the reduction of the activity levels of some of the intracellular protease were part of the death process of *D. abyssinica* after herbicide application. It was also concluded that the combination of reduced dose rates with 2 hand weeding supplements improved the crop performance. The economic analysis done basing on the data obtained from the study showed that the 2 hand weeding supplements combined with fluazifop-butyl (162 g a.i.ha^{-1}) was of convincing advantage over the current weeding practice.

6.7. Future work

This study has opened further research avenues, there is also a necessity to study and understand the biology of *D. abyssinica* for effective control. There is need to assess the weed density from the hand weeding treatment every after each weeding to determine whether the several weedings have an effect on the density of *D. abyssinica*. It is also necessary to assess the effect of the broadleaf weed species on the crop performance prior to the hand weeding supplements. Further investigation on the activity levels of the intracellular protease in the treated plants of *D. abyssinica* and cotton is necessary to determine herbicide response differences of these plant species. Closely co-ordinated and collaborative approaches such as biological control of *D. abyssinica* are necessary to investigate so as to minimise the use of chemicals.

CHAPTER SEVEN

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Appendix 2.2. Percentage control of other grass weed species obtained at each site during the 1997/98 season.

Namulonge

Treatment	*Panicum maximum*	*Sorghum halepense*
Fluazifop-butyl 138 g a.i.ha^{-1}	76.5±3.9	79.8±6.2
Fluazifop-butyl 162 g a.i.ha^{-1}	88.8±4.0	92.3±1.7
Fluazifop-butyl 188 g a.i.ha^{-1}	85.0±3.9	83.8±5.7
Sethoxydim 405 g a.i.ha^{-1}	78.5±6.8	85.3±1.7
Sethoxydim 502 g a.i.ha^{-1}	85.0±0.8	87.5±3.2
Sethoxydim 579 g a.i.ha^{-1}	82.8±2.2	84.5±3.4
Significance level	ns	ns

Bukalasa

Treatment	*Panicum maximum*	*Cynodon dactylon*
Fluazifop-butyl 138 g a.i.ha^{-1}	96.8±0.8	94.3±2.1
Fluazifop-butyl 162 g a.i.ha^{-1}	96.0±2.0	95.3±1.8
Fluazifop-butyl 188 g a.i.ha^{-1}	94.0±1.7	93.3±1.4
Sethoxydim 405 g a.i.ha^{-1}	92.8±1.0	94.5±1.0
Sethoxydim 502 g a.i.ha^{-1}	94.3±2.2	93.5±1.7
Sethoxydim 579 g a.i.ha^{-1}	91.3±2.2	94.0±2.2
Significance level	ns	ns

ns – not significant

Appendix 2.2. Percentage control of other grass weed species obtained at each site during the 1997/98 season.

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Sethoxydim 579 g a.i.ha^{-1}	82.8±2.2	84.5±3.4
Significance level	ns	ns

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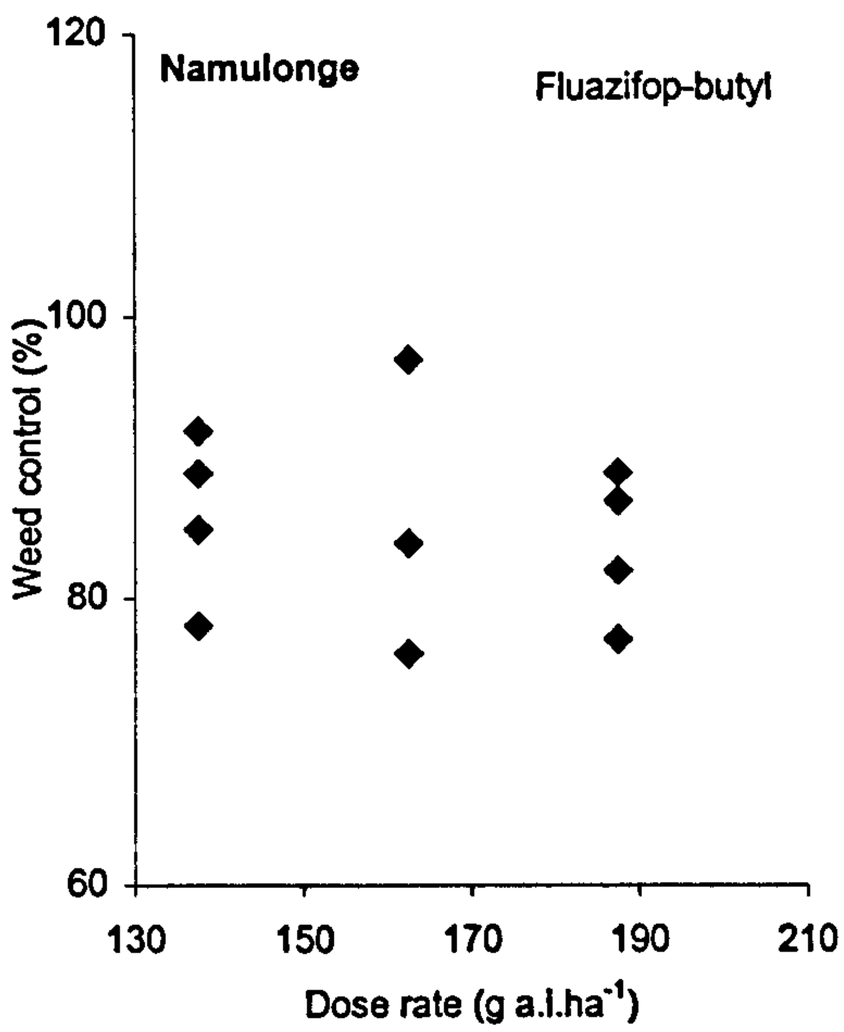
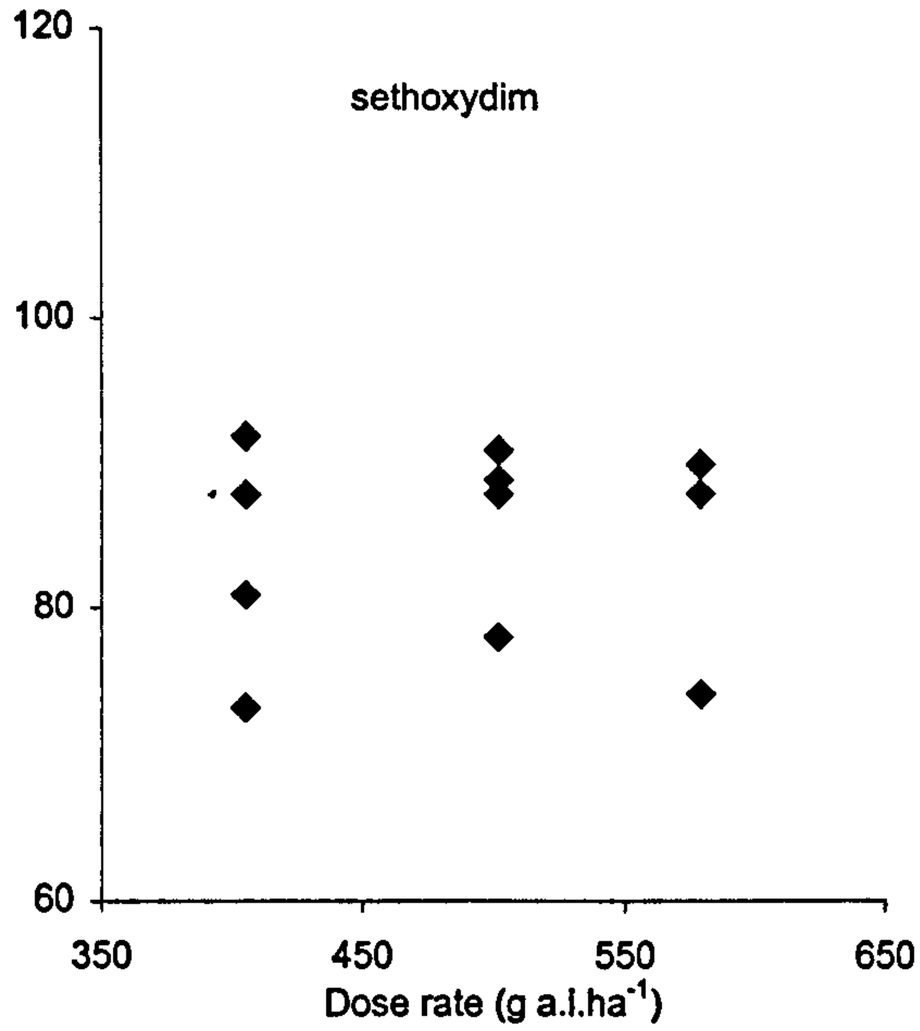
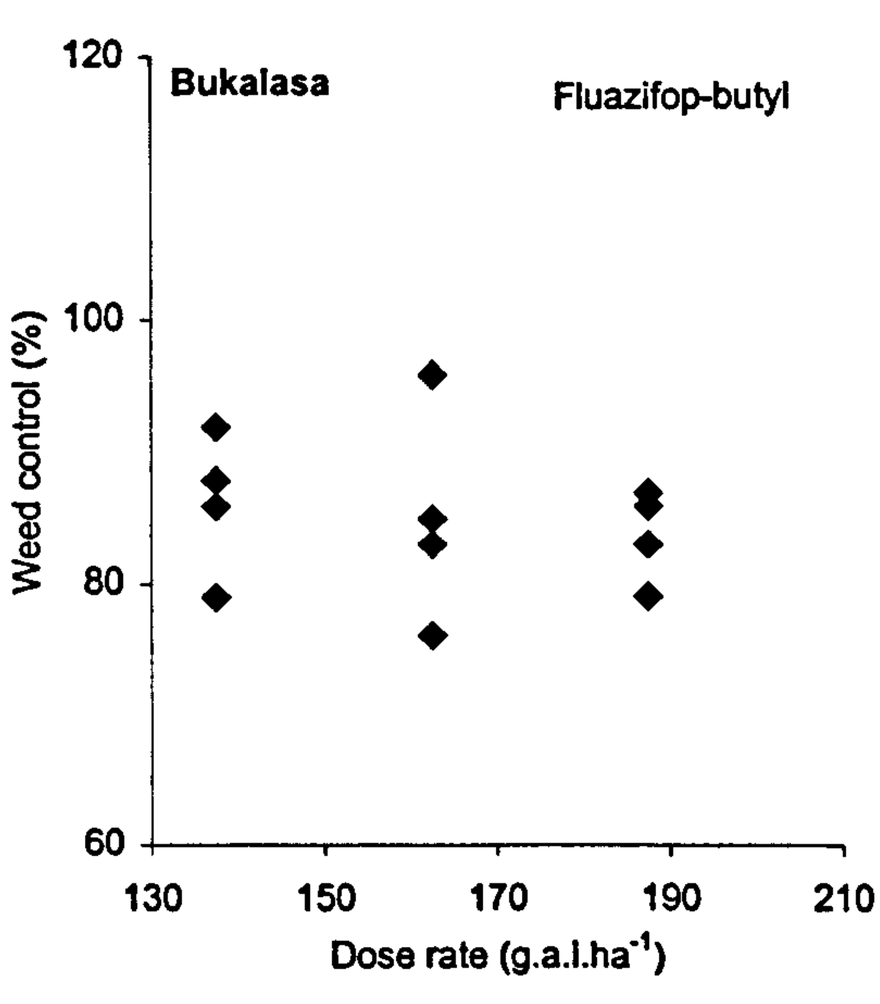
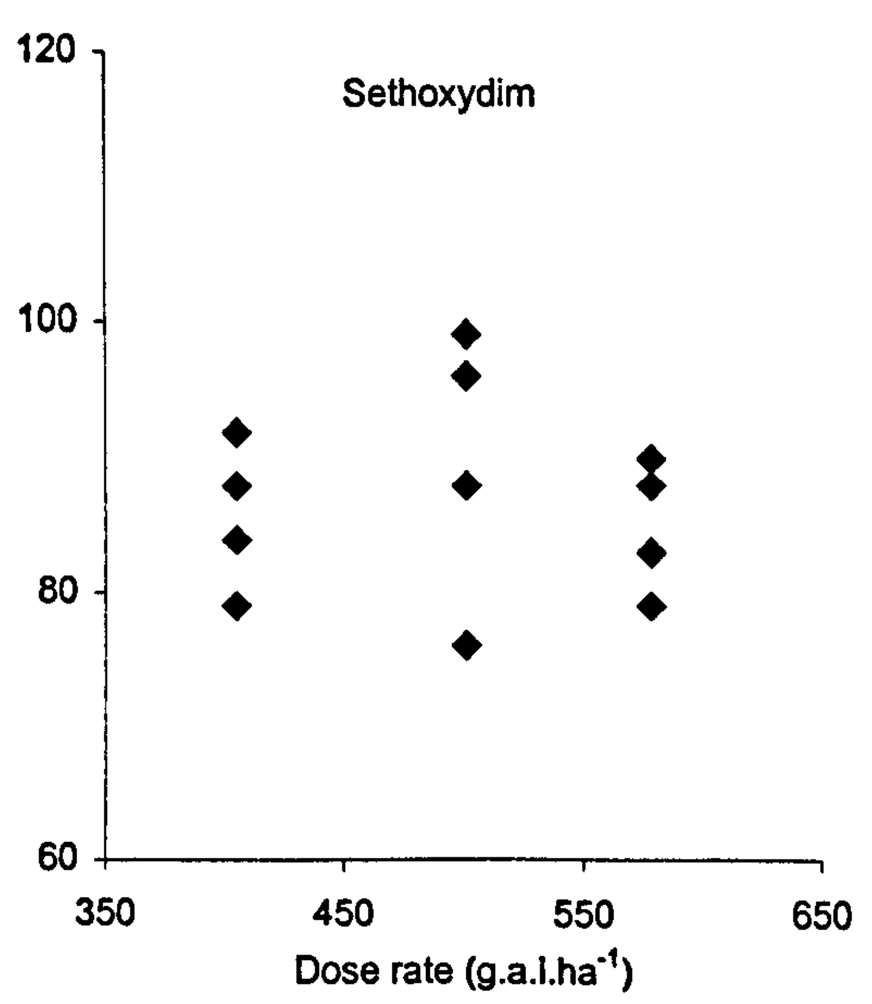
Treatment	*Panicum maximum*	*Cynodon dactylon*
Fluazifop-butyl 138 g a.i.ha^{-1}	96.8±0.8	94.3±2.1
Fluazifop-butyl 162 g a.i.ha^{-1}	96.0±2.0	95.3±1.8
Fluazifop-butyl 188 g a.i.ha^{-1}	94.0±1.7	93.3±1.4
Sethoxydim 405 g a.i.ha^{-1}	92.8±1.0	94.5±1.0
Sethoxydim 502 g a.i.ha^{-1}	94.3±2.2	93.5±1.7
Sethoxydim 579 g a.i.ha^{-1}	91.3±2.2	94.0±2.2
Significance level	ns	ns

ns – not significant

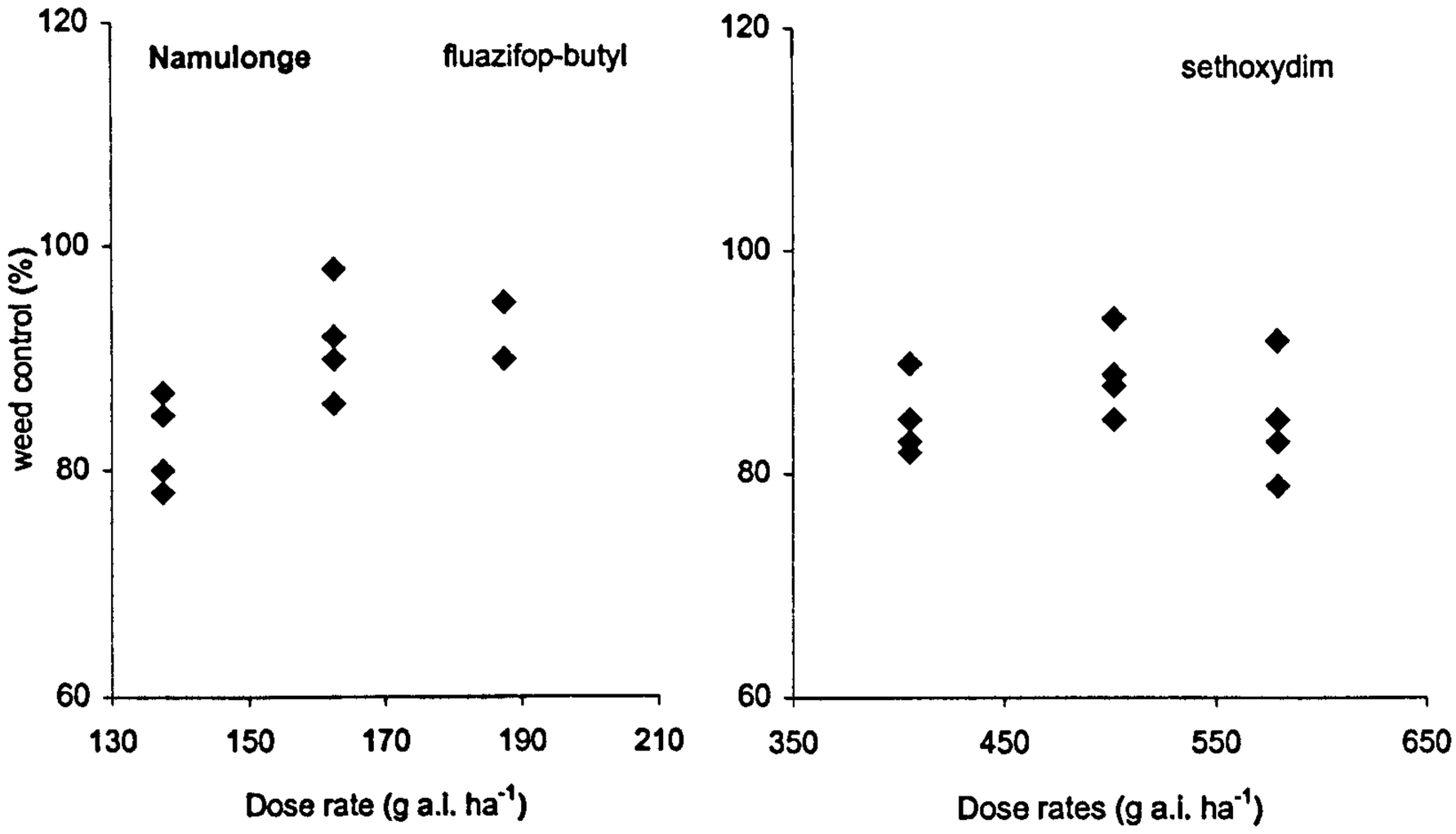




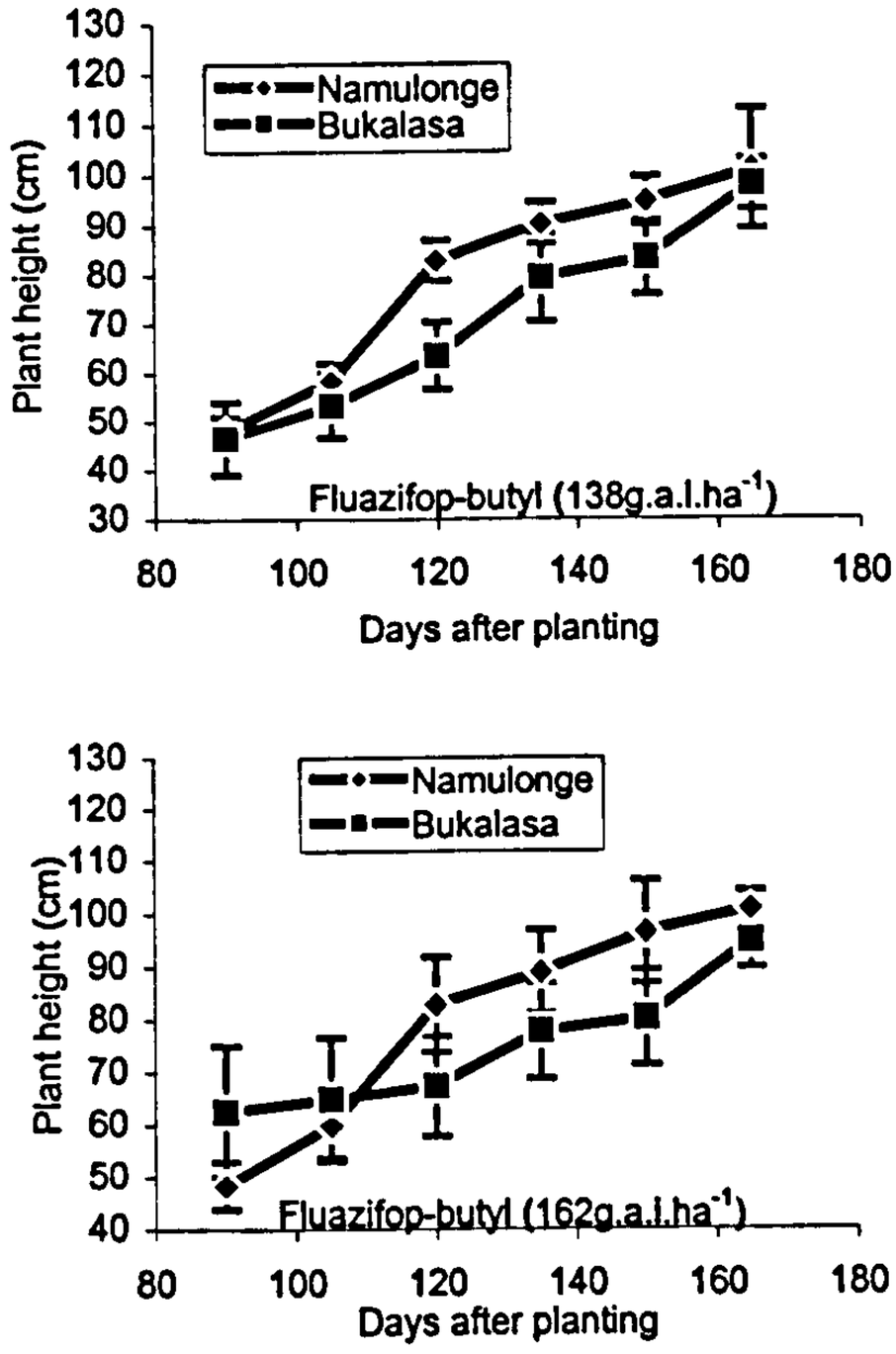


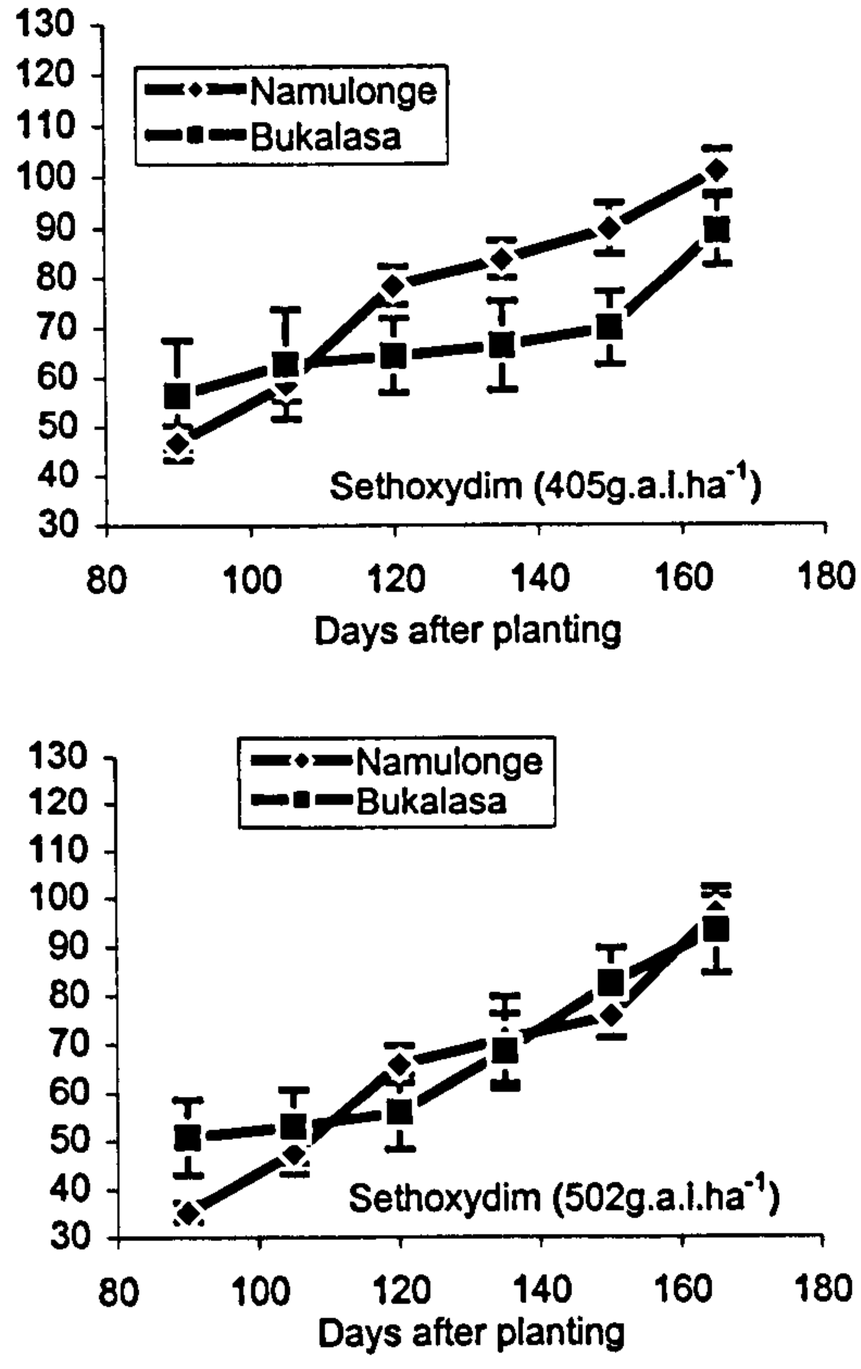


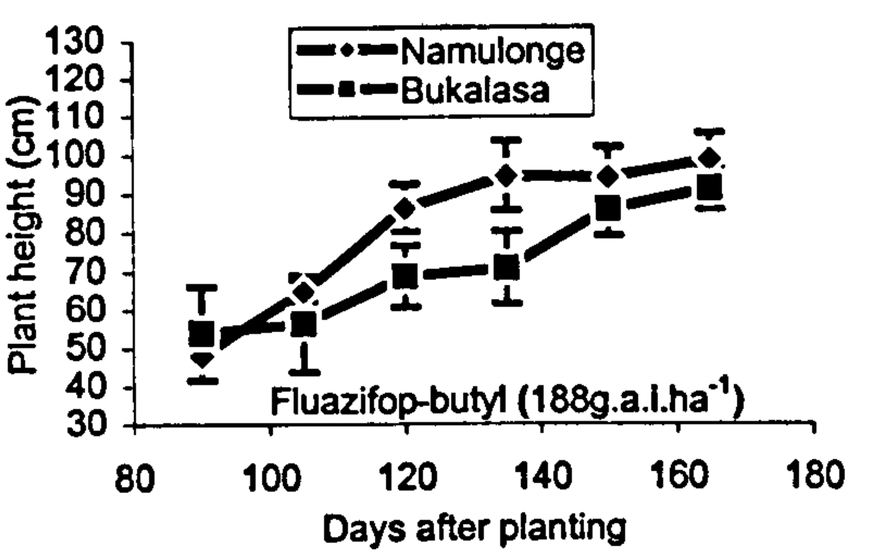
Appendix 2.3. A scatter diagram to show that percentage control of *D. abyssinica* was not dose rate dependant during the 1995/96 season.

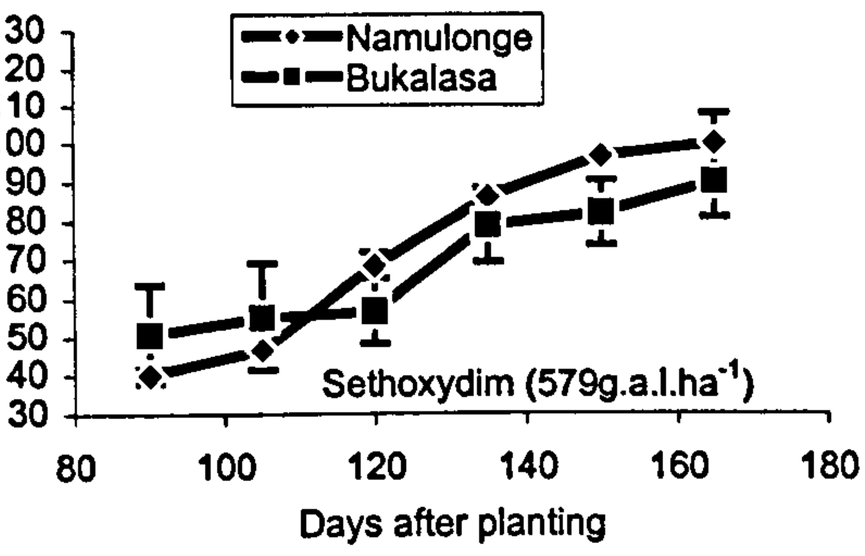


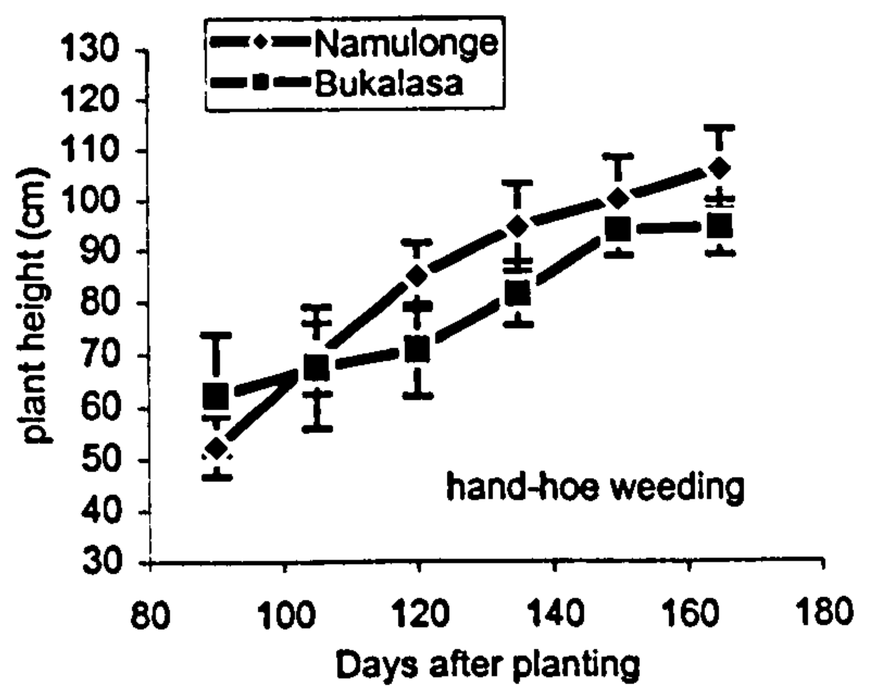
Appendix 2.4. A scatter diagram indicate that percentage control of *D. abyssinica* was not dose rate dependant during the 1997/98 season.

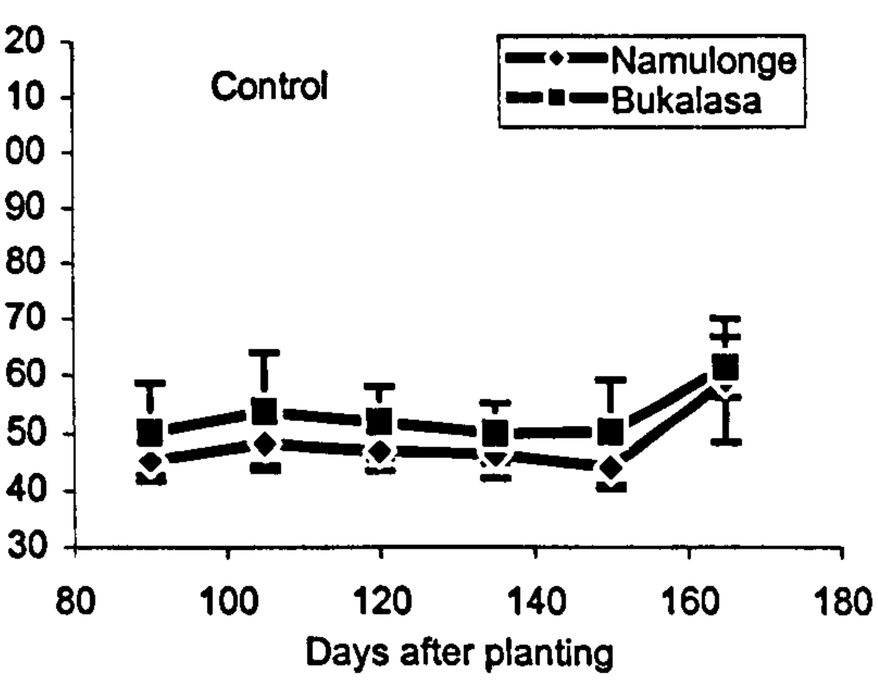




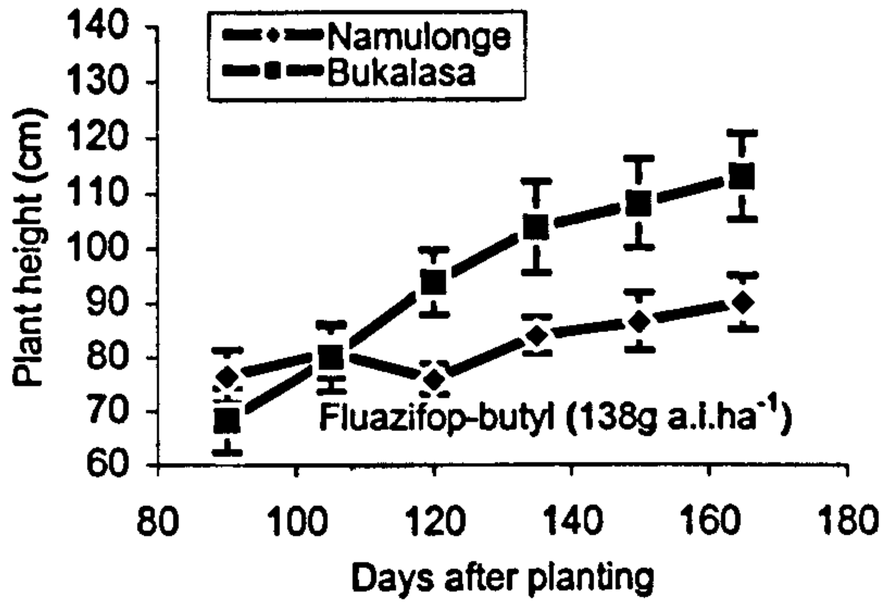


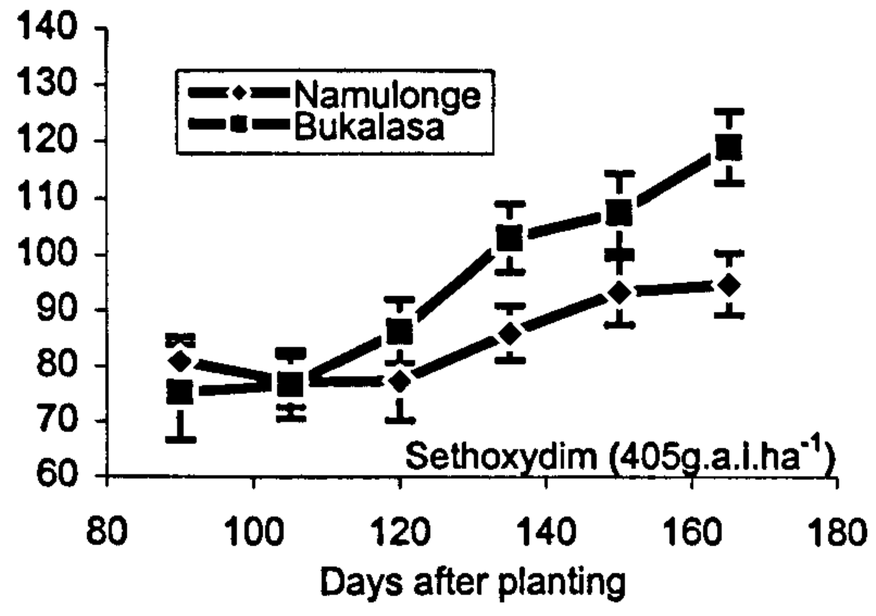


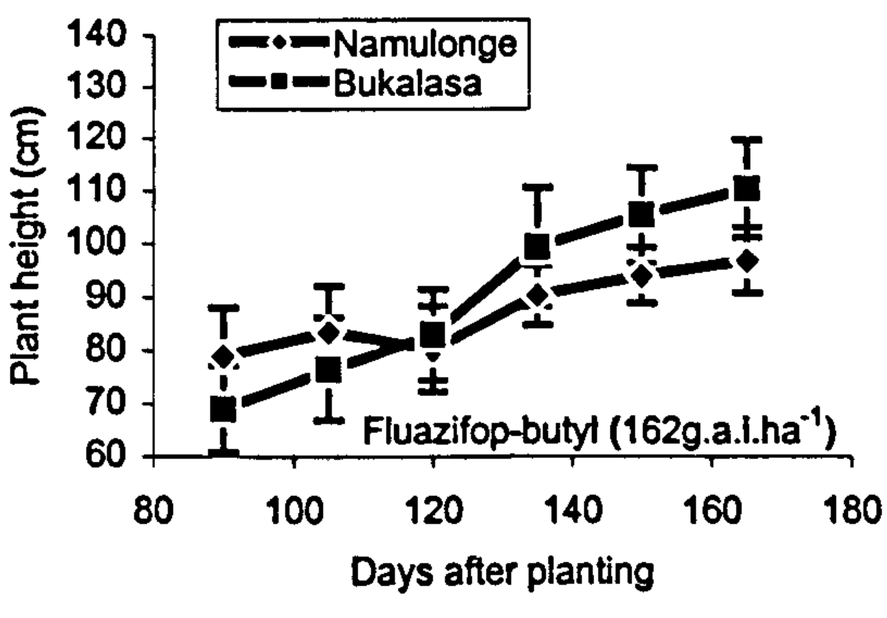


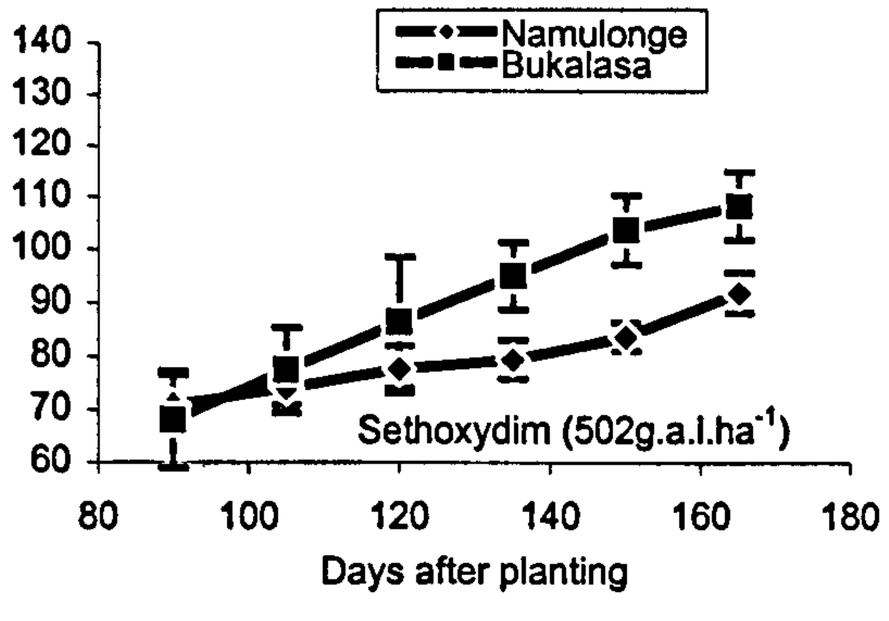


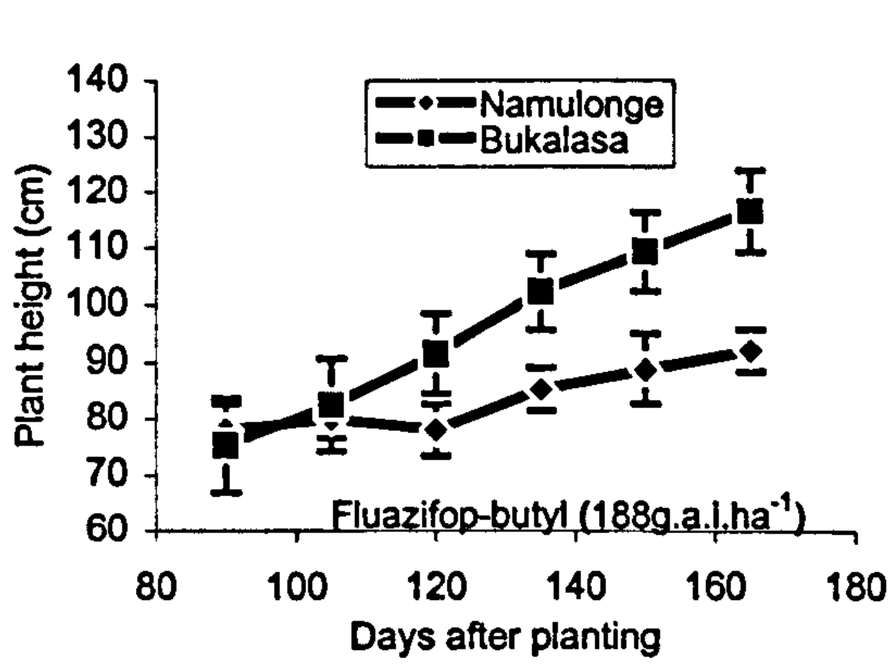
Appendix 2.5. Mean cotton plant height (cm) measured at days after planting during the 1995/96 season.

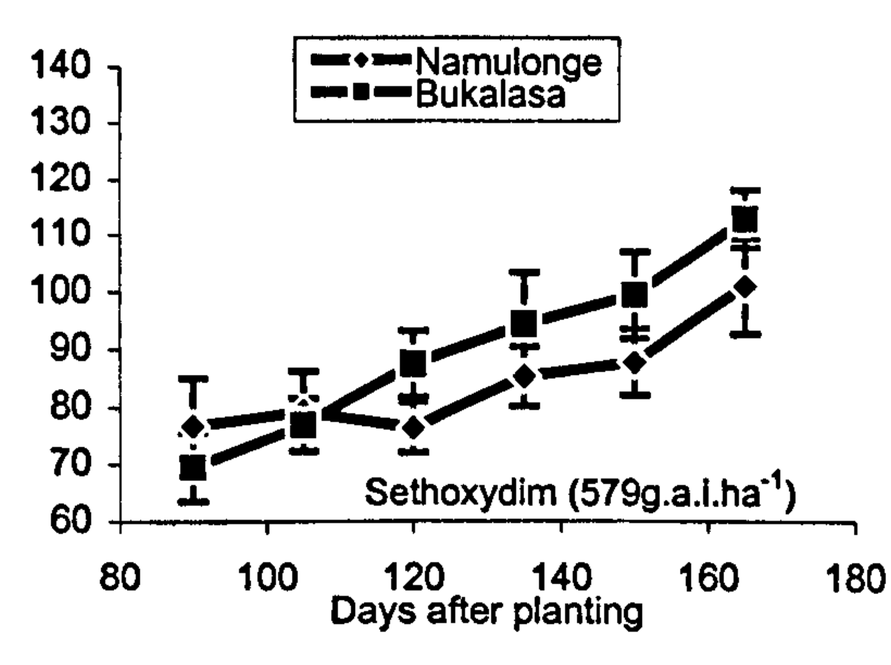


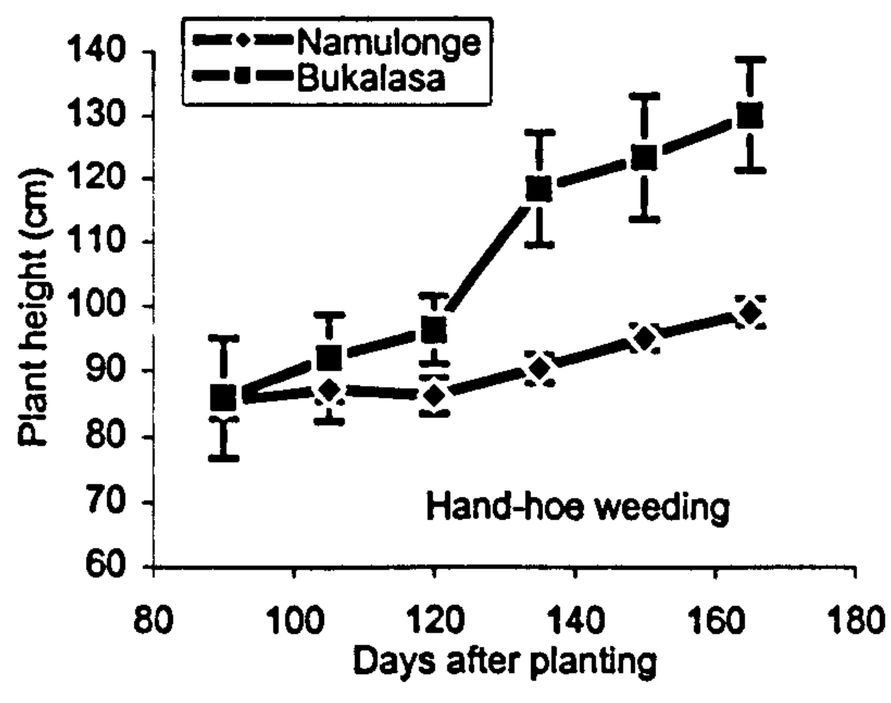


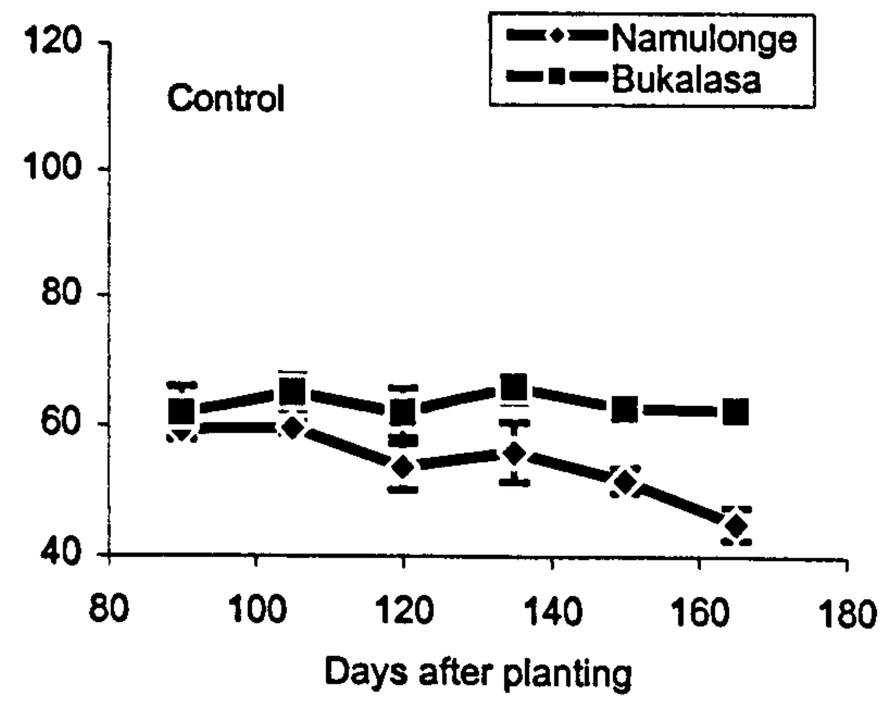




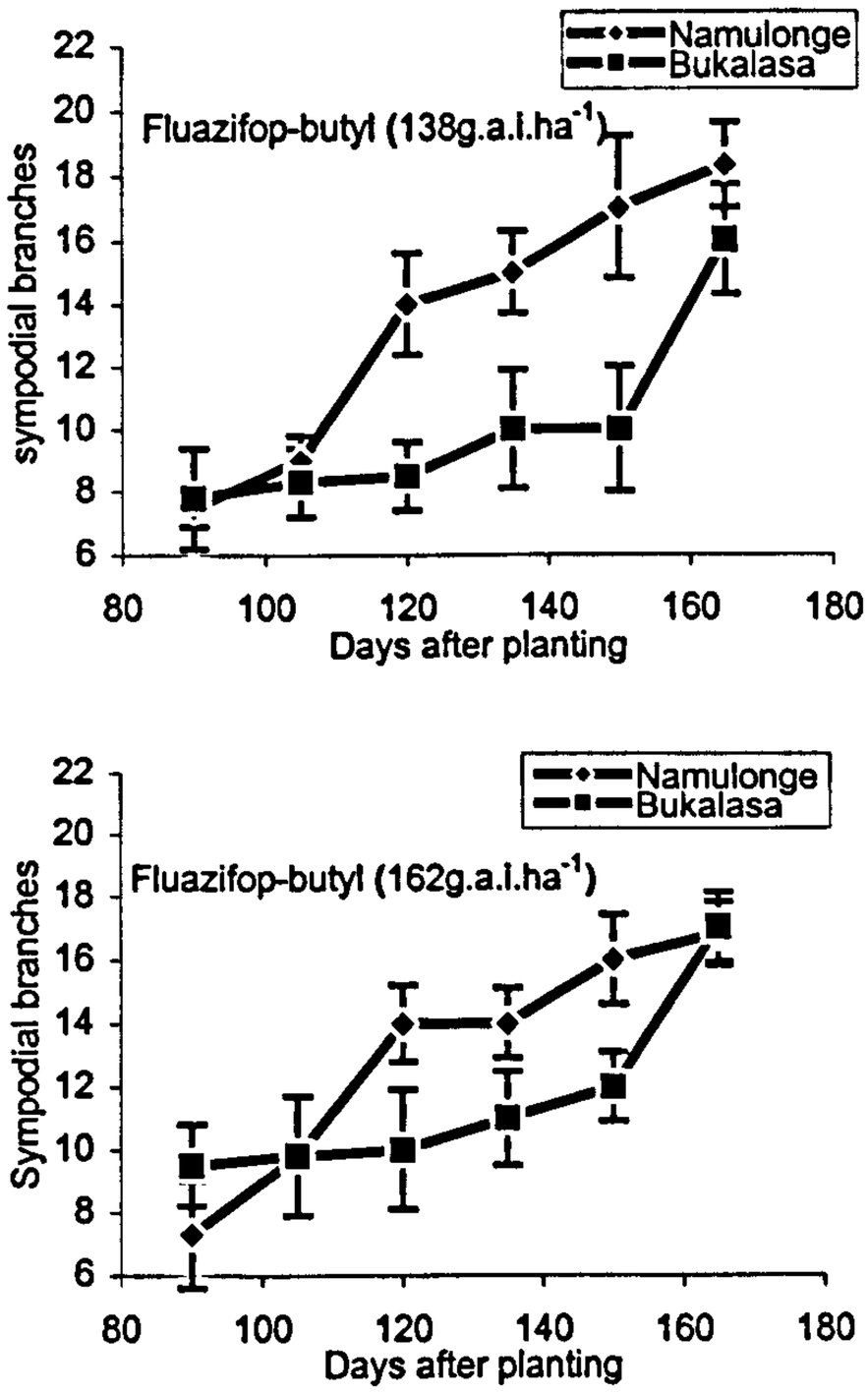


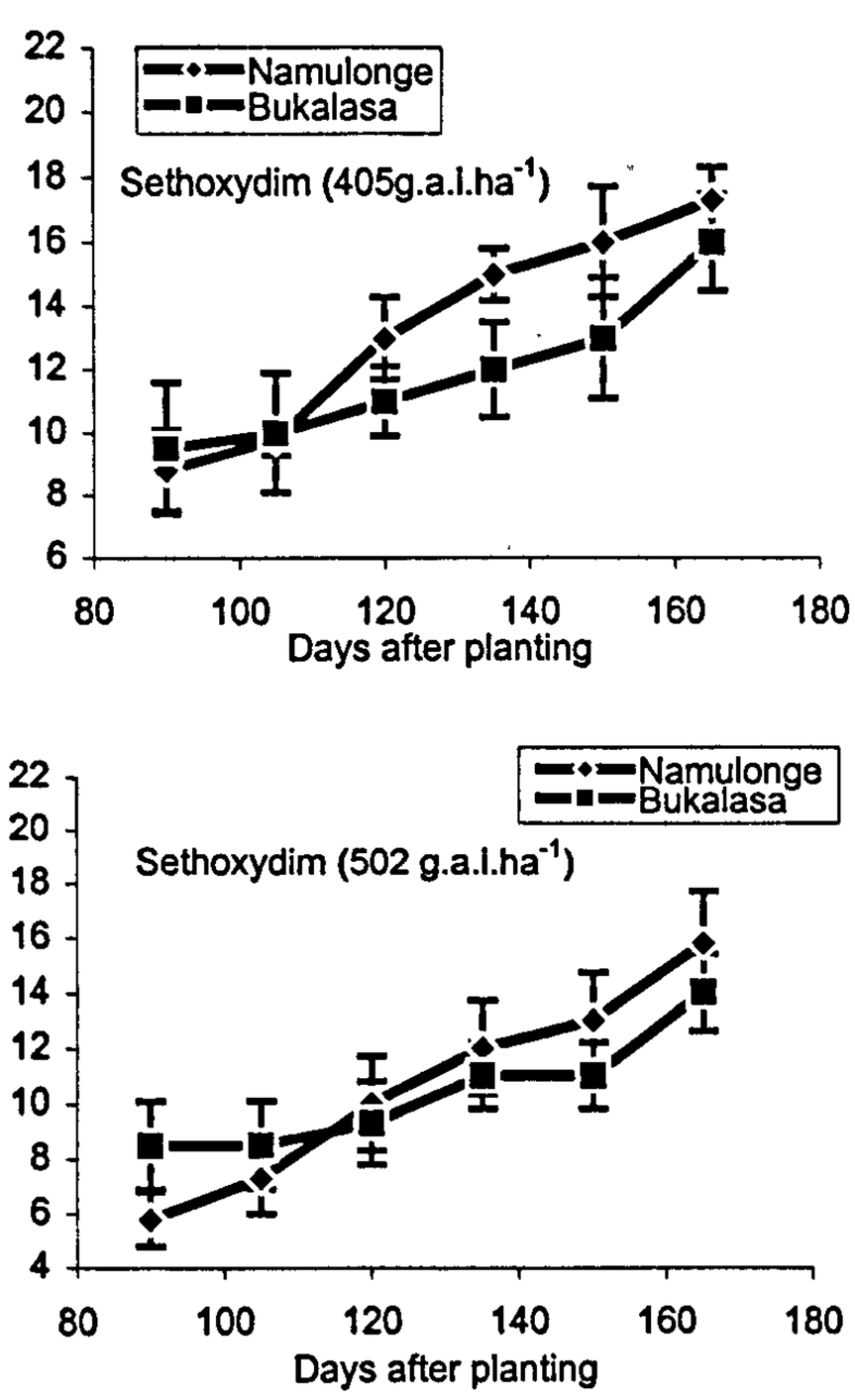


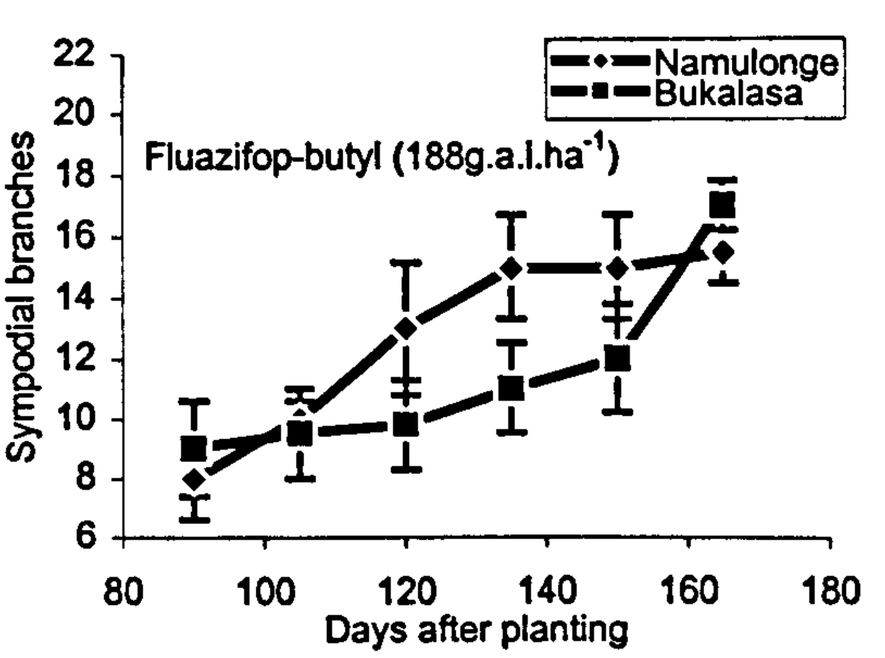


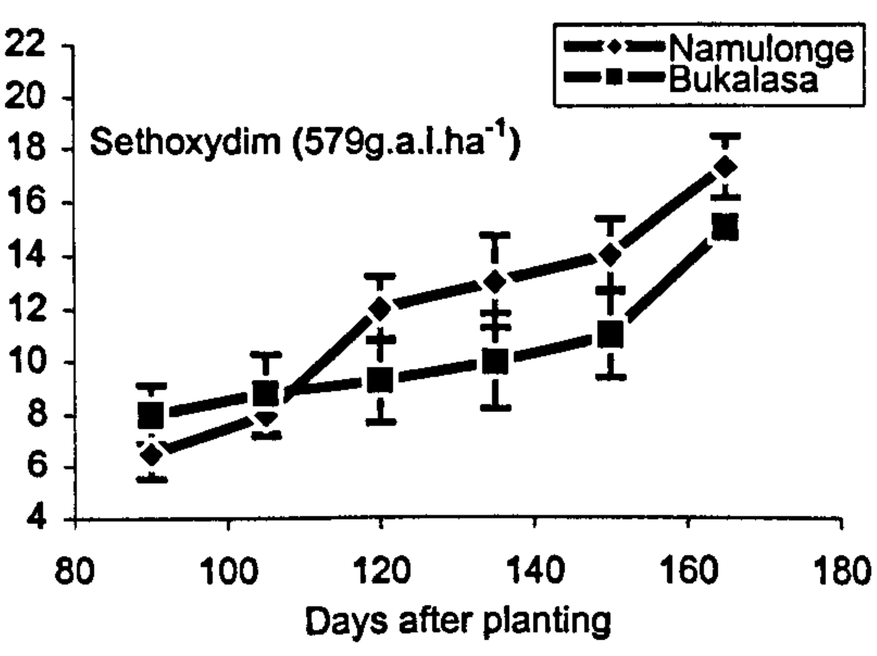


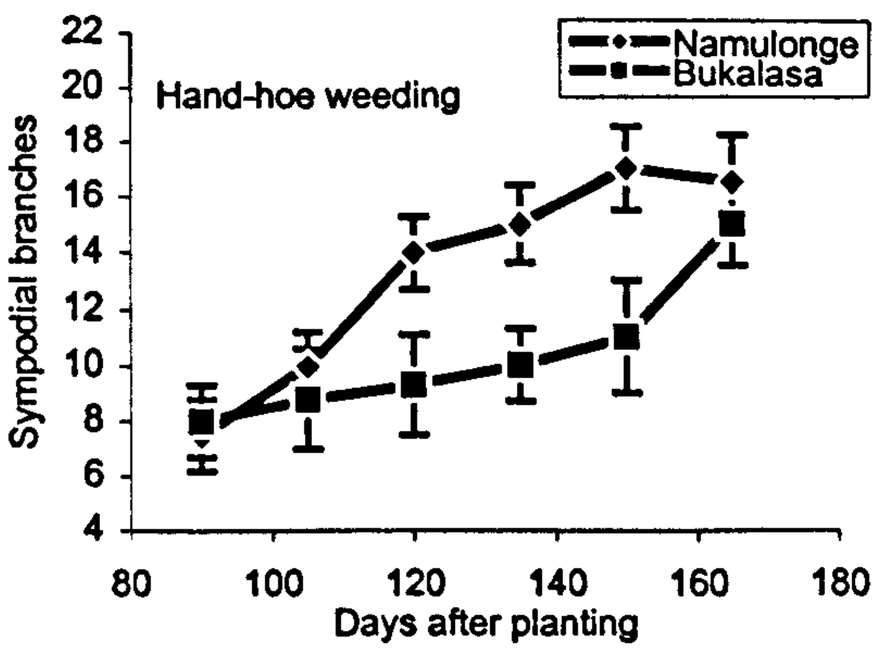
Appendix 2.6. Mean cotton plant height (cm) measured at days after planting during the 1997/98 season.

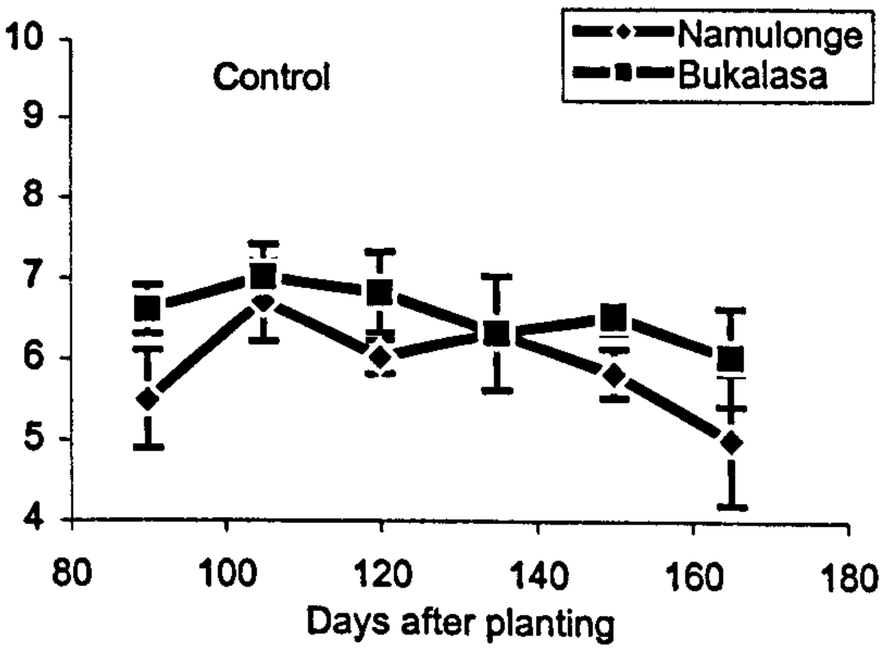




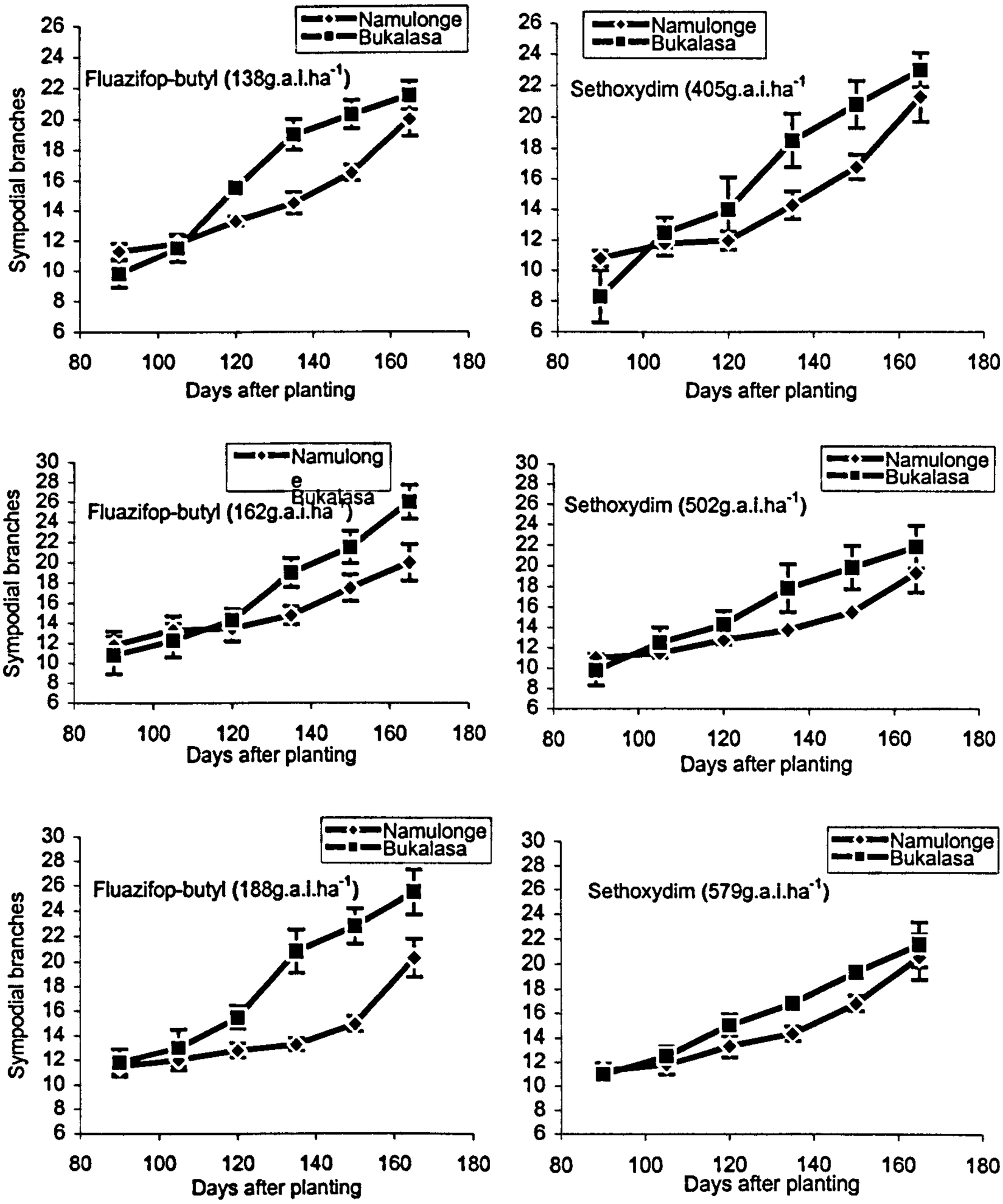




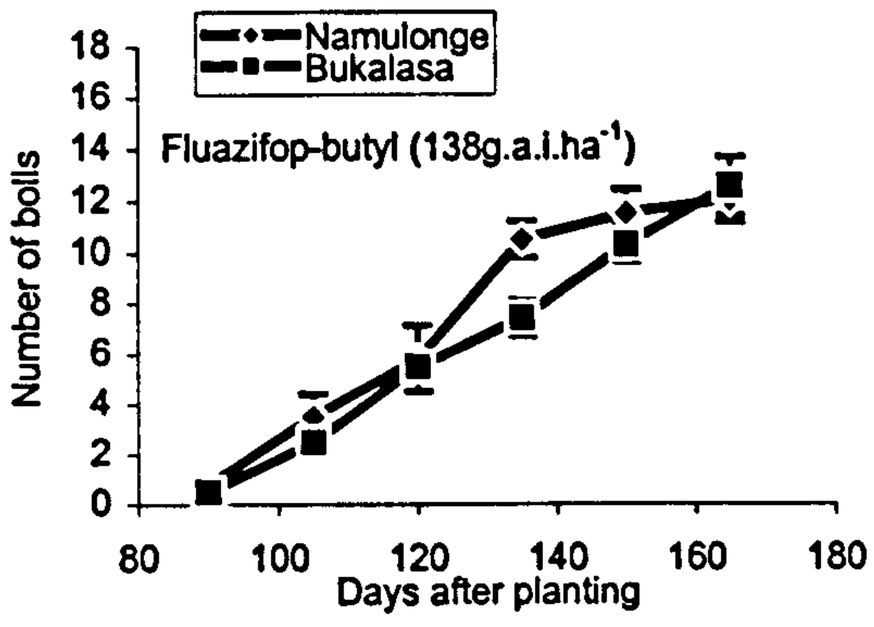


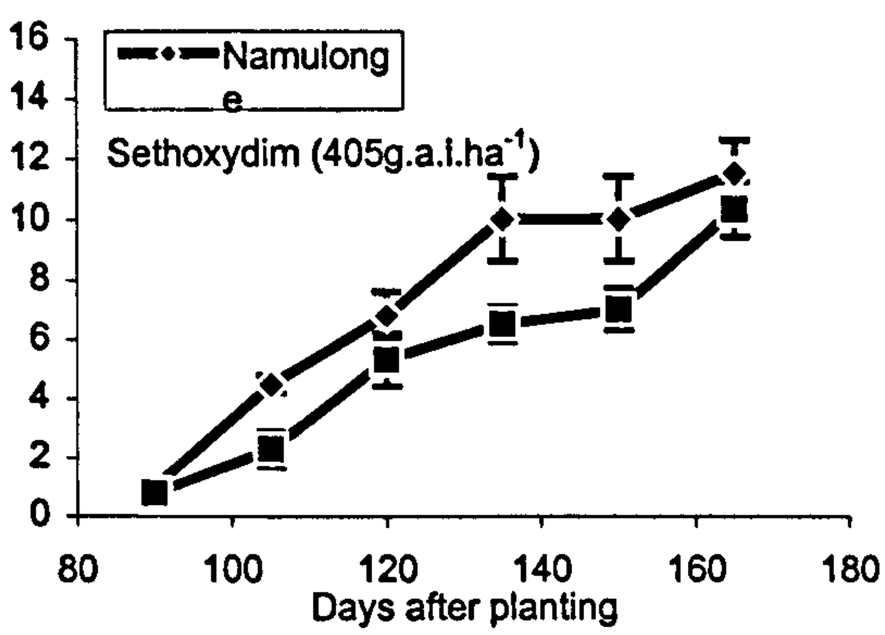


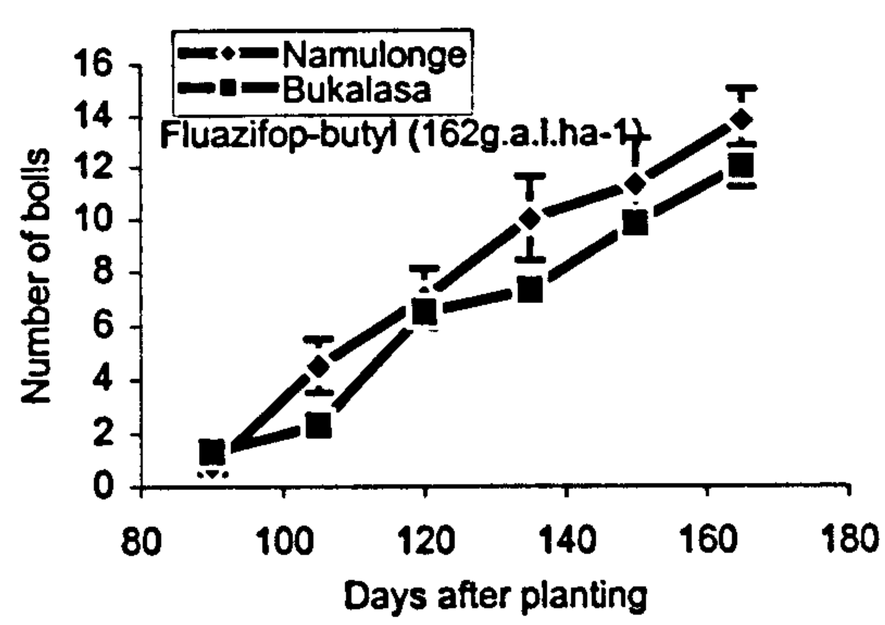
Appendix 2.7. Mean sympodial branches per cotton plant counted at days after planting during the 1995/96 season.

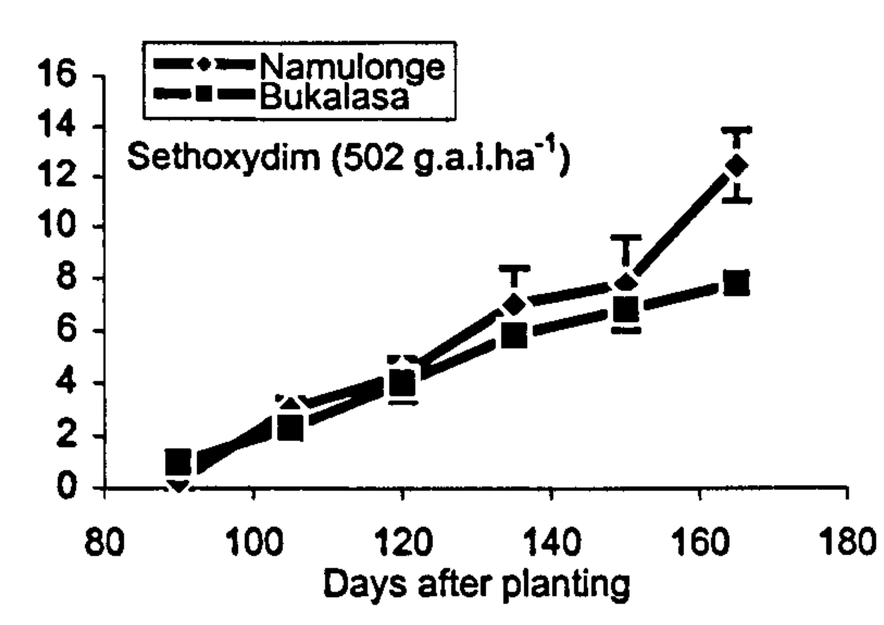


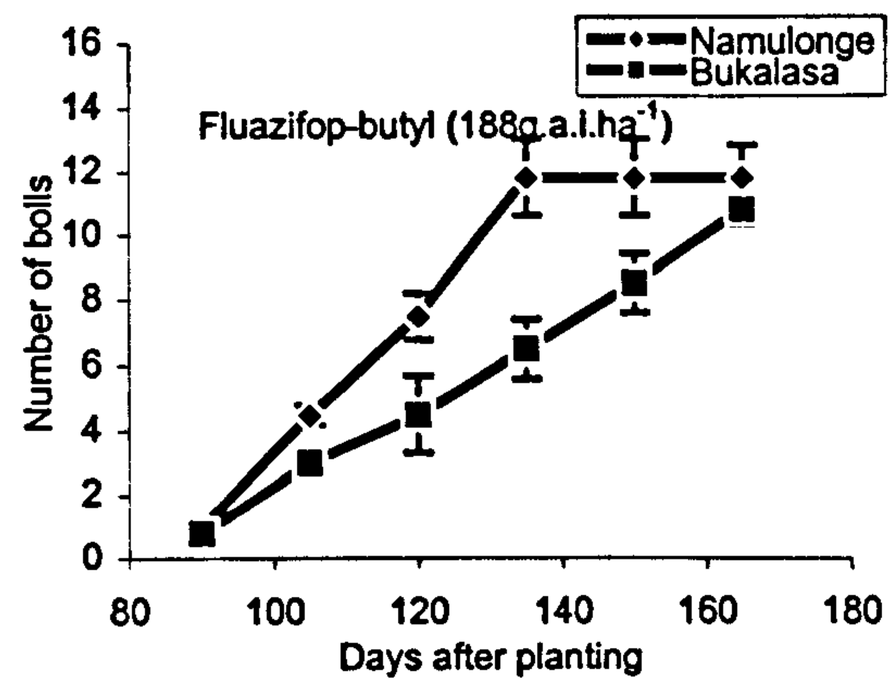
Appendix 2.8. Mean sympodial branches per cotton plant counted at days after planting during the 1997/98 season.

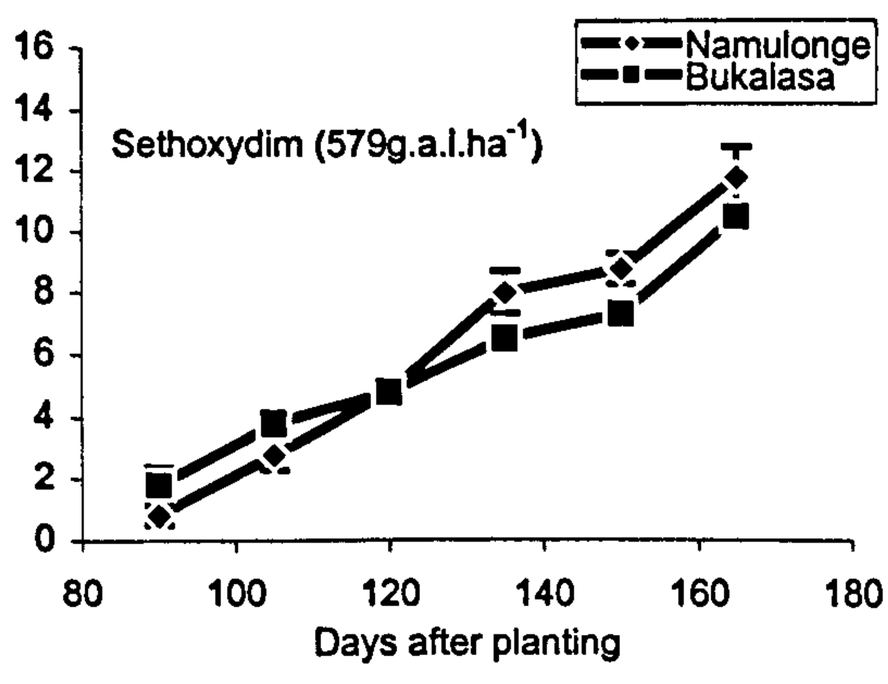


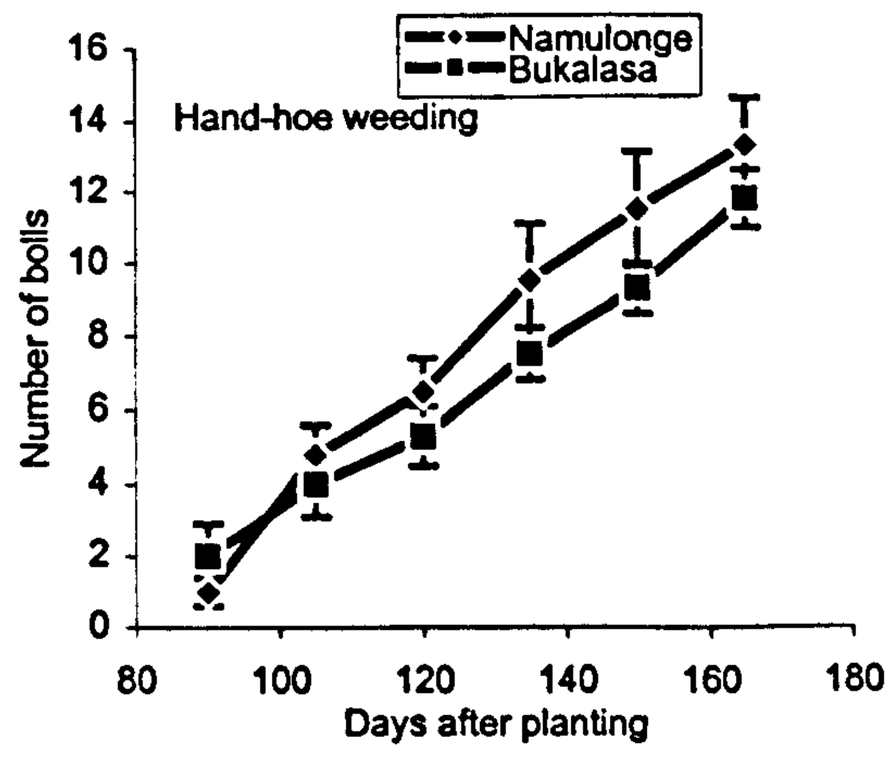


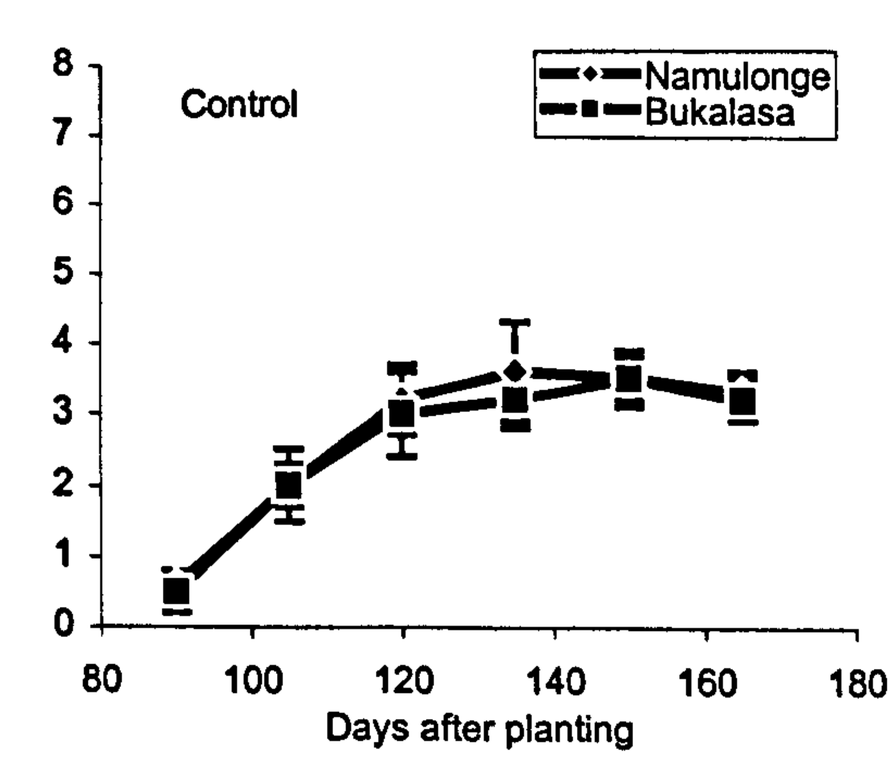




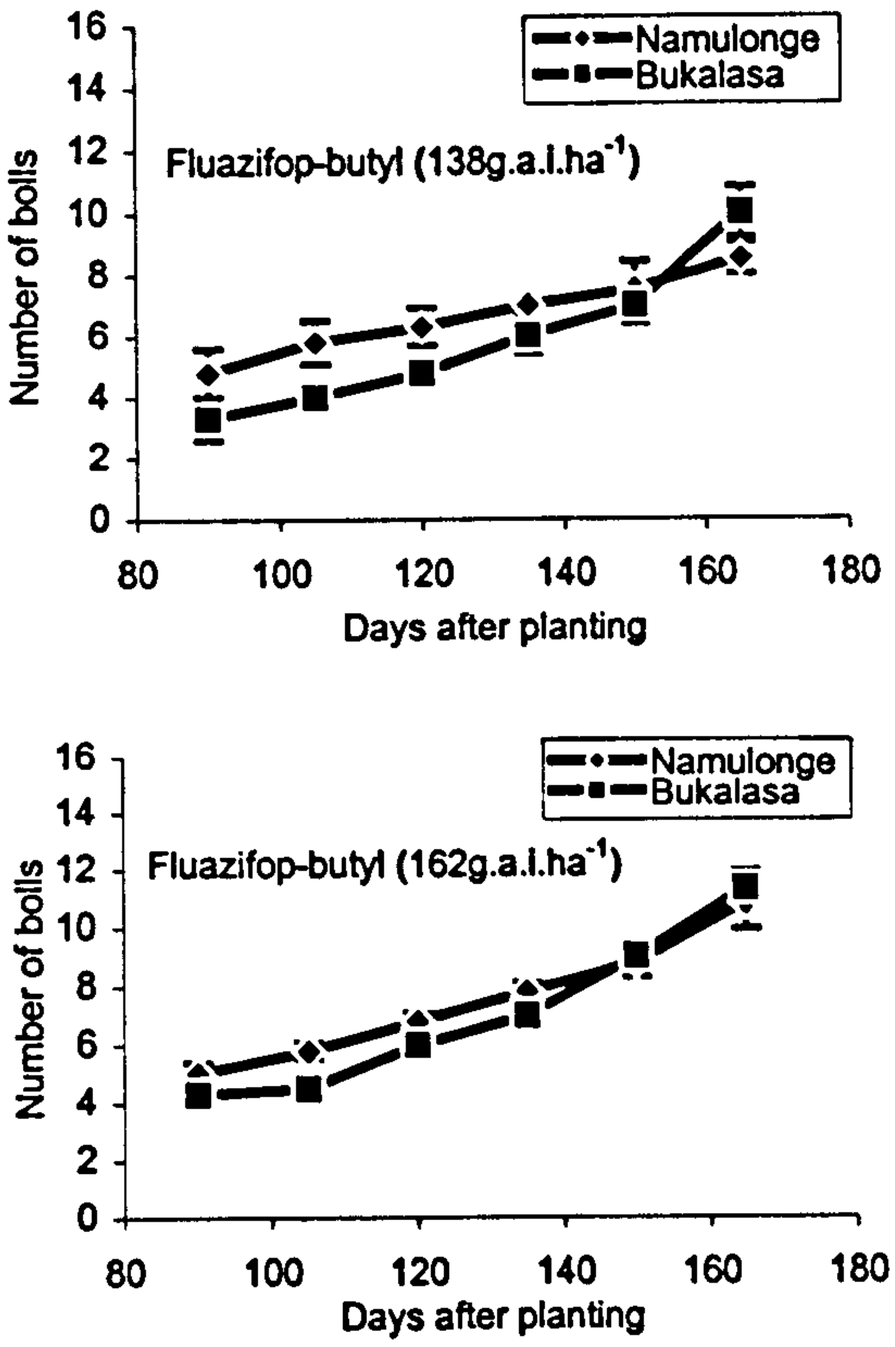


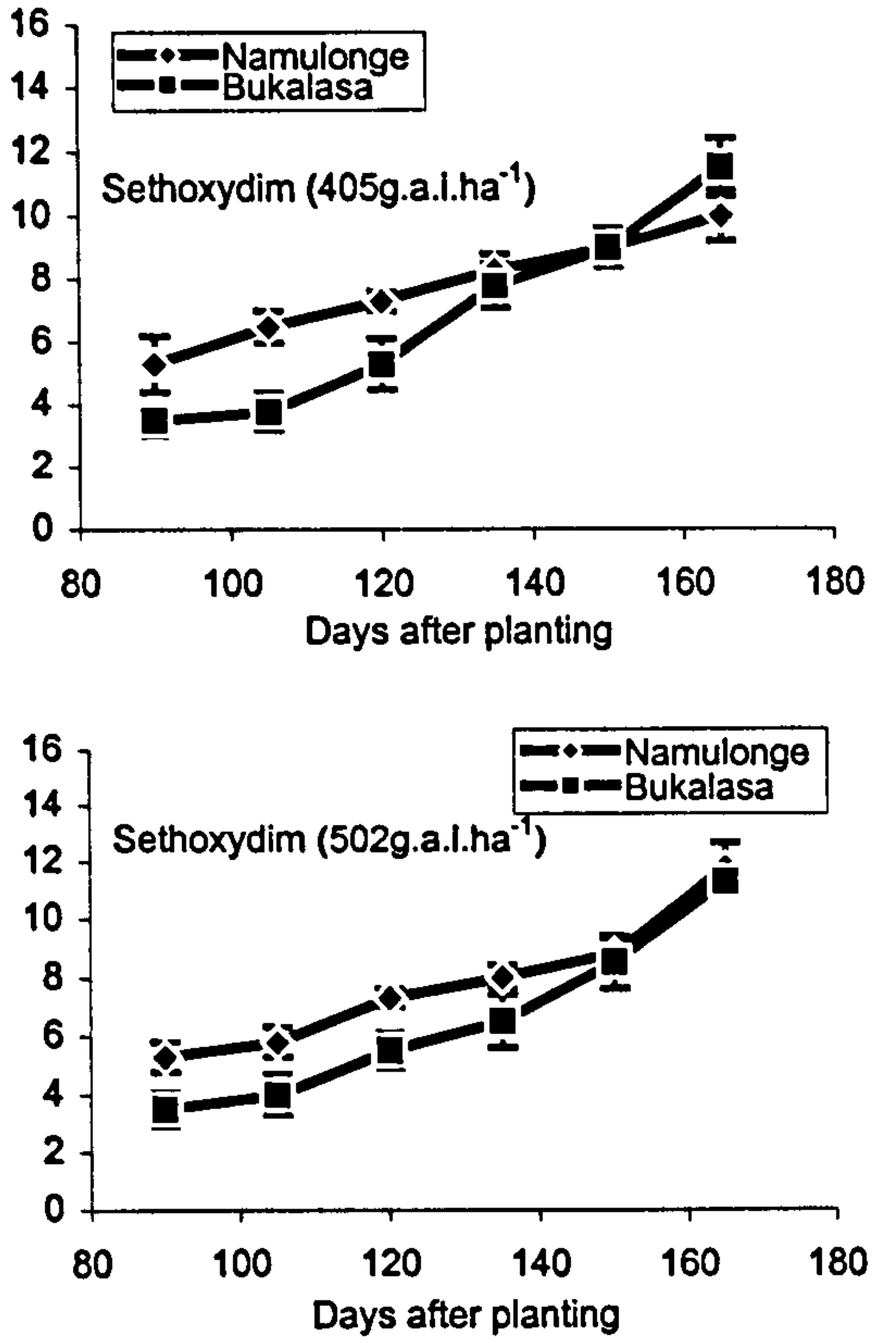


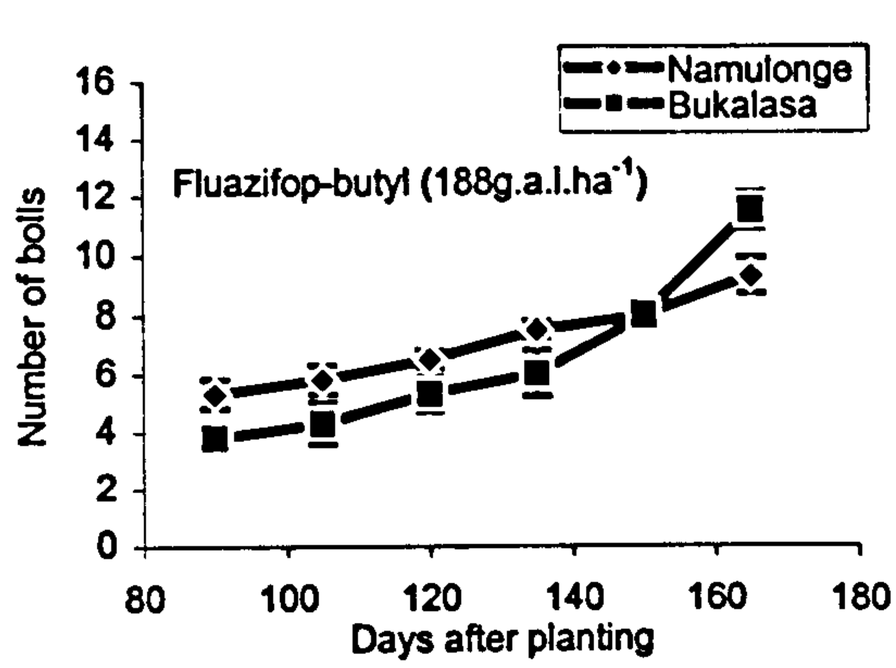


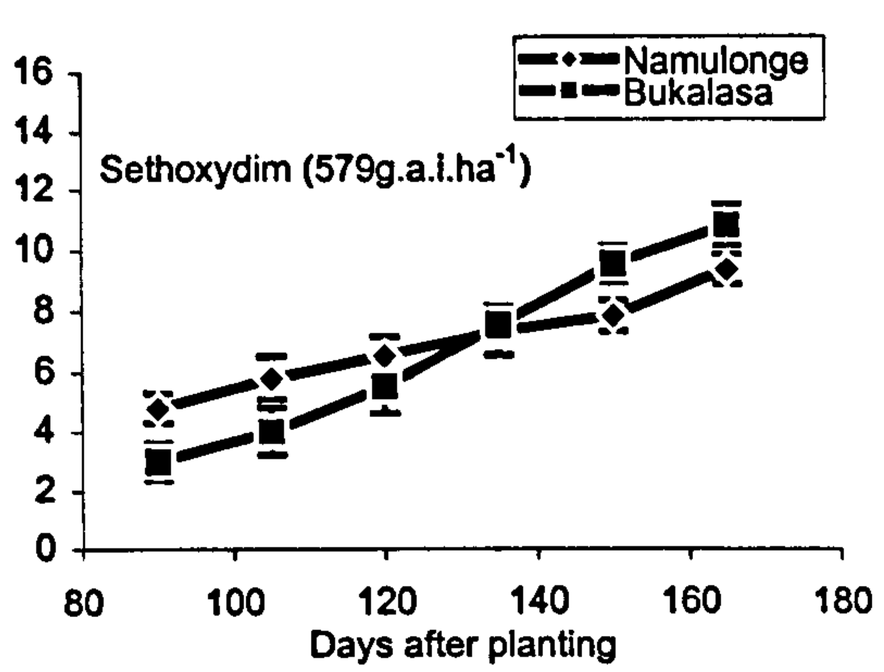


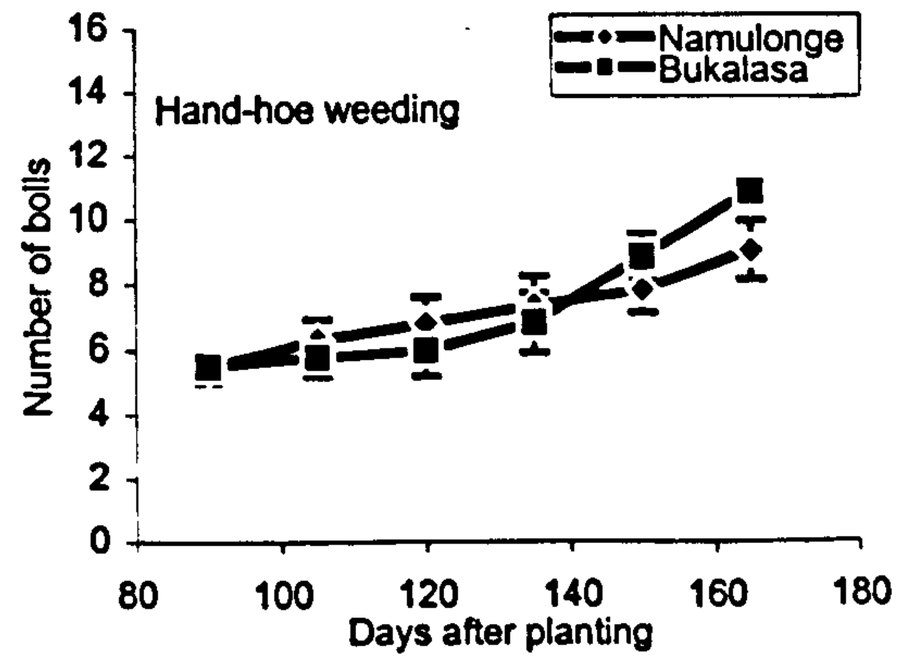
Appendix 2.9. Mean number of bolls per cotton plant counted at days after planting during the 1995/96 season.

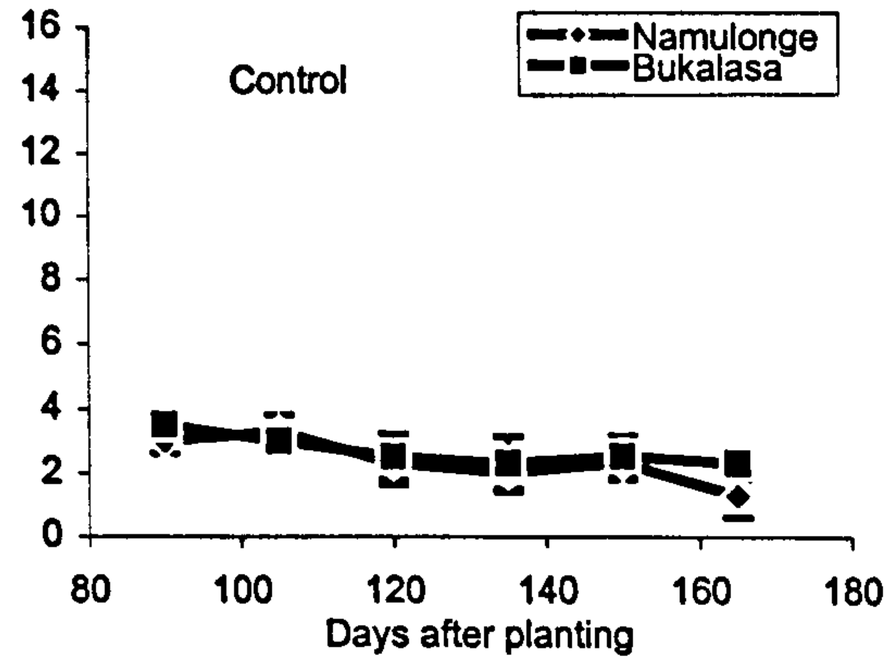












Appendix 2.10. Mean number of bolls per cotton plant counted at days after planting during the 1997/98 season.

Appendix 2.11. Economic analysis of the costs and benefits of weed management in cotton production (Ug shs/kg) at Namulonge and Bukalasa in 1995/96.

Seedcotton yield (kg/ha)

Treatment	Safi kg/ha	Fifi kg/ha	Safi costs shs 350 per kg	Fifi costs shs 150 per kg	Herbicide costs £21.5 /L (shs1750/ pound)	Weeding costs 22 mandays/ha shs 1500/ person	Spraying costs, 3man days/ha, shs. 1500 per person	Sprayer pump hire (shs)	Total costs (Shs)	Gross field revenues (Shs)	Total net benefits (Shs)
Fluazifop-butyl 138g.a.i.ha^{-1}(1.1L)+2hw	1884.3	78.2	659505	11730	41387.5	66000	4500	500	112387.5	671235	558847.5
Fluazifop-butyl 162g.a.i.ha^{-1}(1.3L)+2hw	2833.9	159.8	991865	23970	48912.5	66000	4500	500	119912.5	1015835	895922.5
Fluazifop-butyl 188g.a.i.ha^{-1}(1.5L)+2hw	2559.3	146.9	895755	22035	56437.5	66000	4500	500	127437.5	917790	790352.5
Sethoxydim 405g.a.i.ha^{-1}(2.2L)+2hw	2242.7	82.3	784945	12345	82775	66000	4500	500	153775	797290	643515
Sethoxydim 502g.a.i.ha^{-1}(2.6L)+2hw	2682.4	205.1	938840	30765	97825	66000	4500	500	168825	969605	800780
Sethoxydim 579g.a.i.ha^{-1}(3.0L)+2hw	2601.6	73.4	910560	11010	112875	66000	4500	500	183875	921570	737695
Hand weeding (5 times)	2516	165.2	880600	24780	0	165000	0	0	165000	905380	740380
Control	27	16	9450	2400	0	0	0	0	0	0	11850

Seedcotton yield (kg/ha) **Bukalasa**

Treatment	Safi kg/ha	Fifi kg/ha	Safi costs shs 350 per kg	Fifi costs shs 150 per kg	Herbicide costs £21.5 /L (shs1750/ pound)	Weeding costs 22 mandays/ha shs 1500/ person	Spraying costs, 3man days/ha, shs. 1500 per person	Sprayer pump hire (shs)	Total costs (Shs)	Gross field revenues (Shs)	Total net benefits (Shs)
Fluazifop-butyl 138g.a.i.ha^{-1}(1.1L)+2hw	1757.4	36.3	615090	5445	41387.5	66000	4500	500	112387.5	620535	508147.5
Fluazifop-butyl 162g.a.i.ha^{-1}(1.3L)+2hw	2167.8	201	758730	30150	48912.5	66000	4500	500	119912.5	788880	668967.5
Fluazifop-butyl 188g.a.i.ha^{-1}(1.5L)+2hw	2121.9	59.1	742665	8865	56437.5	66000	4500	500	127437.5	751530	624092.5
Sethoxydim 405g.a.i.ha^{-1}(2.2L)+2hw	2004.2	95.8	701470	14370	82775	66000	4500	500	153775	715840	562065
Sethoxydim 502g.a.i.ha^{-1}(2.6L)+2hw	2217.9	19.6	776265	2940	97825	66000	4500	500	168825	779205	610380
Sethoxydim 579g.a.i.ha^{-1}(3.0L)+2hw	2085.5	27	729925	4050	112875	66000	4500	500	183875	733975	550100
Hand weeding (5 times)	2175	300	761250	45000	0	165000	0	0	165000	806250	641250
Control	16.5	31	5775	4650	0	0	0	0	0	0	10425

Appendix 2.12. Economic analysis of the costs and benefits of weed management in cotton production (Ug.shs/kg) at Namulonge and Bukalasa in 1997/98

Seedcotton yield (kg/ha) **Namulonge**

Treatment	Safi kg/ha	Fifi kg/ha	Safi costs shs 350 per kg	Fifi costs shs 150 per kg	Herbicide costs £21.5 /L (shs2175/ pound)	Weeding costs 22 mandays/ha, shs 1500/ person	Spraying costs, 3man days/ha, shs. 1500 per person	Sprayer pump hire (shs)	Total costs (Shs)	Gross field revenues (shs)	Total net benefits (Shs)
Fluazifop-butyl 138g.a.i.ha^{-1}(1.1L)+2hw	607	58	212590	8625	51439	66000	4500	500	122439	221215	98776
Fluazifop-butyl 162g.a.i.ha^{-1}(1.3L)+2hw	817	99	286020	14775	60791	66000	4500	500	131791	300795	169004
Fluazifop-butyl 188g.a.i.ha^{-1}(1.5L)+2hw	833	75	291655	11295	70144	66000	4500	500	141144	302950	161806
Sethoxydim 405g.a.i.ha^{-1}(2.2L)+2hw	684	20	239295	3060	102878	66000	4500	500	173878	242355	68478
Sethoxydim 502g.a.i.ha^{-1}(2.6L)+2hw	987	15	345555	2280	121583	66000	4500	500	192583	347835	155253
Sethoxydim 579g.a.i.ha^{-1}(3.0L)+2hw	984	24	344225	3540	140288	66000	4500	500	211288	347765	136478
Hand weeding (5 times)	815	83	285320	12420	0	165000	0	0	165000	297740	132740
Control	20	12	7035	1725	0	0	0	0	0	0	8760

Seedcotton yield (kg/ha) **Bukalasa**

Treatment	Safi kg/ha	Fifi kg/ha	Safi costs shs 350 per kg	Fifi costs shs 150 per kg	Herbicide costs £21.5 /L (shs2175/ pound)	Weeding costs 22 mandays/ha, shs 1500/ person	Spraying costs, 3man days/ha, shs. 1500 per person	Sprayer pump hire (shs)	Total costs (Shs)	Gross field revenues (shs)	Total net benefits (Shs)
Fluazifop-butyl 138g.a.i.ha^{-1}(1.1L)+2hw	844	47	295295	7110	51439	66000	4500	500	122439	302405	179966
Fluazifop-butyl 162g.a.i.ha^{-1}(1.3L)+2hw	1095	89	383320	13350	60791	66000	4500	500	131791	396670	264879
Fluazifop-butyl 188g.a.i.ha^{-1}(1.5L)+2hw	825	56	288575	8415	70144	66000	4500	500	141144	296990	155846
Sethoxydim 405g.a.i.ha^{-1}(2.2L)+2hw	707	39	247275	5850	102878	66000	4500	500	173878	253125	79248
Sethoxydim 502g.a.i.ha^{-1}(2.6L)+2hw	820	39	287070	5865	121583	66000	4500	500	192583	292935	100353
Sethoxydim 579g.a.i.ha^{-1}(3.0L)+2hw	924	18	323365	2685	140288	66000	4500	500	211288	326050	114763
Hand weeding (5 times)	848	72	296905	10725	0	165000	0	0	165000	307630	142630
Control	11	37	3885	5475	0	0	0	0	0	0	9360

Appendix 2.13. Experimental field layout during the 1995/96 and 1997/98 seasons at Namulonge and Bukalasa.

1 **T1**	16 **T8**	17 **T7**	32 **T6**
2 **T6**	15 **T5**	18 **T3**	31 **T8**
3 **T2**	14 **T7**	19 **T4**	30 **T1**
4 **T4**	13 **T1**	20 **T8**	29 **T3**
5 **T5**	12 **T6**	21 **T2**	28 **T4**
6 **T3**	11 **T2**	22 **T5**	27 **T7**
7 **T7**	10 **T3**	23 **T6**	26 **T5**
8 **T8**	9 **T4**	24 **T1**	25 **T2**
Rep 1	**Rep 2**	**Rep 3**	**Rep 4**

Numbers 1-32 represents plot numbers

Rep – Replication

T – Treatment

Appendix 3.1. Analysis of variance for the percentage reduction of the fresh *D. abyssinica* shoots.

Source	DF	SS	MS	F	P
Treatment	6	247.23	41.20	8.19**	0.000
Error	35	176.11	5.03		
Total	41	423			

** = significant at 1%

Appendix 3.2. Analysis of variance for the percentage reduction of the dry *D. abyssinica* shoots.

Source	DF	SS	MS	F	P
Treatment	6	16.449	2.741	3.06*	0.016
Error	35	31.4	0.897		
Total	41	47.848			

* = significant at 5%

Appendix 3.3. Analysis of variance for the percentage reduction of the fresh *Digitaria* rhizomes.

Source	DF	SS	MS	F	P
Treatment	6	871.86	145.31	17.29**	0.000
Error	35	294.09	8.40		
Total	41	1165.95			

** = significant at 1%

Appendix 3.4. Analysis of variance for the percentage reduction of the dry *Digitaria* rhizomes.

Source	DF	SS	MS	F	P
Treatment	6	28.120	4.687	10.40**	0.000
Error	35	15.770	0.451		
Total	41	43.891			

** = significant at 1%

Appendix 3.5. Analysis of variance for minimum fluorescence of *D. abyssinica* after herbicide application.

Source	DF	SS	MS	F	P
Treatment	6	720117	120020	21.13**	0.000
Error	21	1199303	5681		
Total	27	839420			

** = significant at 1%

Appendix 3.6. Analysis of variance for Fv/Fm of *D. abyssinica* after herbicide application.

Source	DF	SS	MS	F	P
Treatment	6	0.5278	0.0880	8.34**	0.000
Error	21	0.2215	0.0105		
Total	27	0.7493			

** = significant at 1%

Appendix 4.1. Analysis of variance for the percentage reduction of the fresh shoots of *D. abyssinica*.

Source	DF	SS	MS	F	P
Treatment	8	14109	1764	5.65**	0.000
Error	45	14040	312		
Total	53	28149			

** = significant at 1%

Appendix 4.2. Analysis of variance for the percentage reduction of the dry shoots of *D. abyssinica*.

Source	DF	SS	MS	F	P
Treatment	8	10356	1294	5.04**	0.000
Error	45	11547	257		
Total	53	21903			

** = significant at 1%

Appendix 4.3. Analysis of variance for the percentage reduction of the fresh rhizomes of *D. abyssinica*.

Source	DF	SS	MS	F	P
Treatment	8	28290	3536	19.64**	0.000
Error	45	8102	180		
Total	53	36391			

** = significant at 1%

Appendix 4.4. Analysis of variance for the percentage reduction of the dry rhizomes of *D. abyssinica*.

Source	DF	SS	MS	F	P
Treatment	8	24676	3085	14.59**	0.000
Error	45	9515	211		
Total	53	34192			

** = significant at 1%

Appendix 4.5. Analysis of variance for the reduction of chlorophyll content of *D. abyssinica* leaves.

Source	DF	SS	MS	F	P
Treatment	8	1498.18	187.27	42.96**	0.000
Error	45	196.18	4.36		
Total	53	1694.36			

** = significant at 1%

Appendix 5.1.Analysis of Variance for protein content of cotton and *D. abyssinica.*

Source	DF	SS	MS	F	P
Time	2	543495	271748	1.62	0.239
Trt	1	539139	539139	3.21	0.098
spp	1	16073065	16073065	95.73	0.000**
Time x Trt	2	14549	7274	0.04	0.958
Time x spp	2	718876	359438	2.14	0.160
Trt x spp	1	2683393	2683393	15.98	0.002**
Time x Trt x spp	2	161591	80796	0.48	0. 629
Error	12	2014889	167907		
Total	23	22748998			

spp= Cotton and *D. abyssinica*

Appendix 5.2. Analysis of Variance for the activity level of Ala Ap in *D. abyssinica* and cotton.

Source	DF	SS	MS	F	P
Time	2	17997	8998	0.92	0.424
Trt	1	9106	9106	0.93	0.353
spp	1	3088708	3088708	316.48	0.000**
TimexTrt	2	2614	1307	0.13	0.876
Timexspp	2	60904	30452	3.12	0.081
Trtxspp	1	10279	10279	1.05	0.325
TimexTrtxspp	2	6683	3341	0.34	0.717
Error	12	117114	9760		
Total	23	3313404			

Appendix 5.3. Analysis of Variance for the activity level of Arginyl-ap in *D. abyssinica* and cotton.

Source	DF	SS	MS	F	P
Time	2	7483	3741	2.82	0.099
Trt	1	2591	2591	1.95	0.188
spp	1	262826	262826	198.04	0.000**
TimexTrt	2	11373	5686	4.28	0.039*
Timexspp	2	19849	9925	7.48	0.008**
Trtxspp	1	33	33	0.02	0.878
TimexTrtxspp	2	192	96	0.07	0.931
Error	12	15926	1327		
Total	23	320273			

Appendix 5.4. Analysis of Variance for the activity level of DAP 4 in cotton and *D. abyssinica.*

Source	DF	SS	MS	F	P
Time	2	7.83	3.92	0.45	0.646
Trt	1	12.63	12.63	1.46	0.250
spp	1	1313.21	1313.21	152.19	0.000**
TimexTrt	2	44.07	22.03	2.55	0.119
Timexspp	2	141.17	70.59	8.18	0.006**
Trtxspp	1	31.30	31.30	3.63	0.081
TimexTrtxspp	2	26.29	13.15	1.52	0.257
Error	12	103.54	8.63		
Total	23	1680.05			

Appendix 5.5.Analysis of Variance for the activity level of TAPin cotton and D. abyssinica.

Source	DF	SS	MS	F	P
Time	2	11309	5654	11.12	0.002**
Trt	1	2679	2679	5.27	0.041*
spp	1	403497	403497	793.27	0.000**
TimexTrt	2	2766	1383	2.72	0.106
Timexspp	2	16531	8266	16.25	0.000**
Trtxspp	1	4965	4965	9.76	0.009**
TimexTrtxspp	2	743	871	1.71	0.222
Error	12	6104	509		
Total	23	449594			

Appendix 5.6.Analysis of Variance for the activity level of DAP1 in cotton and *D. abyssinica*.

Source	DF	SS	MS	F	P
Time	2	488.9	244.4	18.42	0.000**
Trt	1	0.1	0.1	0.01	0.943
spp	1	28072.0	28072.0	2115.76	0.000**
TimexTrt	2	27.3	13.6	1.03	0.387
Timexspp	2	1000.6	500.3	37.71	0.000**
Trtxspp	1	7.1	7.1	0.54	0.477
TimexTrtxspp	2	6.4	3.2	0.24	0.791
Error	12	159.2	13.3		
Total	23	29761.5			

Appendix 5.7. Analysis of Variance for the activity level of DAP2 in cotton and *D. abyssinica*.

Source	DF	SS	MS	F	P
Time	2	17.959	8.979	2.90	0.094
Trt	1	11.708	11.708	3.78	0.076
spp	1	615.956	615.956	198.67	0.000**
TimexTrt	2	20.129	10.065	3.25	0.075
Timexspp	2	23.367	11.683	3.77	0.054
Trtxspp	1	8.927	8.927	2.88	0.115
TimexTrtxspp	2	21.182	10.591	3.42	0.067
Error	12	37.205	3.100		
Total	23	756.433			

Appendix 5.8. Analysis of Variance for the activity level of CathB+L in cotton and *D. abyssinica*.

Source	DF	SS	MS	F	P
Time	2	577.5	288.8	5.24	0.023*
Trt	1	19.2	19.2	0.35	0.566
spp	1	48957.9	48957.9	887.99	0.000**
TimexTrt	2	131.9	65.9	1.20	0.336
Timexspp	2	1867.1	933.6	16.93	0.000**
Trtxspp	1	156.6	156.6	2.84	0.118
TimexTrtxspp	2	337.9	169.0	3.06	0.084
Error	12	661.6	55.1		
Total	23	52709.8			

Appendix 5.9. Analysis of Variance for the activity level of CathB in cotton and *D. abyssinica*.

Source	DF	SS	MS	F	P
Time	2	634.2	317.1	13.08	0.001**
Trt	1	37.3	37.3	1.54	0.239
spp	1	51268.0	51268.0	2114.88	0.000**
TimexTrt	2	0.9	0.4	0.02	0.982
Timexspp	2	1081.8	540.9	22.31	0.000**
Trtxspp	1	96.3	96.3	3.97	0.069
TimexTrtxspp	2	3.2	1.6	0.07	0.937
Error	12	290.9	24.2		
Total	23	53412.5			

Appendix 5.10. Raw data on the soluble protein content of cotton and *D. abyssinica* measured at times after the application of sethoxydim.

Rep	Time	Trt	spp	protein
1	2	579	g	708.52
1	2	0	g	849.44
1	8	579	g	726.47
1	8	0	g	869.7
1	48	579	g	477.1
1	48	0	g	1020.96
1	2	579	c	2146.71
1	2	0	c	1874
1	8	579	c	3195.75
1	8	0	c	1825.48
1	48	579	c	3078.36
1	48	0	c	1078.37
2	2	579	g	783.34
2	2	0	g	1093.87
2	8	579	g	575.45
2	8	0	g	1115.58
2	48	579	g	382.56
2	48	0	g	917.85
2	2	579	c	2578.25
2	2	0	c	1470.49
2	8	579	c	3419.43
2	8	0	c	2713.03
2	48	579	c	3067.77
2	48	0	c	2713.81

g=grass(*D. abyssinica*) c=cotton

Appendix 5.11. Raw data on the protease activities measured in the grass (*D. abyssinica*) and cotton frozen leaves at collected at times after the application of sethoxydim.

Rep	Time (h)	Trt	spp	Ala Ap	Arginyl-AP	DAP 4	TAP	DAP1	DAP2	CathB+L	CathB
1	2		579 g	494.5161	235.4839	13.11828	26.12903	15.4902	3.627451	27.45098	23.82353
1	2		0 g	540.7407	207.284	15.06173	9.012346	10.33898	3.135593	20.59322	20.59322
1	8		579 g	584	194.6667	16.26667	9.733333	13.85965	3.245614	32.01754	24.5614
1	8		0 g	580.4819	175.9036	19.03614	29.27711	17.54237	3.135593	20.59322	23.72881
1	48		579 g	530.9091	152.6364	6.636364	22.09091	6.239316	3.162393	17.69231	20.76923
1	48		0 g	674.557	249.4937	15.44304	30.75949	10.79646	3.274336	18.31858	18.31858
1	2		579 c	1116.9	343.1	28	243.3	76.7	24.3	88.8	101
1	2		0 c	1314	365	24.3	243.3	76.7	12.2	118	113.2
1	8		579 c	1314	416.1	28	292	69.4	12.2	97.3	97.3
1	8		0 c	1262.9	438	30.4	243.3	73	12.2	101	104.6
1	48		579 c	1460	438	28	389.3	88.8	12.2	125.4	121.7
1	48		0 c	1387	562.1	32.9	284.7	93.7	12.2	118	125.4
2	2		579 g	661.5625	228.125	12.70833	25.3125	12.34375	2.890625	28.51563	21.875
2	2		0 g	789.4186	195.2326	14.18605	36.74419	10.42735	3.162393	23.93162	20.76923
2	8		579 g	703.6145	202.2892	14.6988	29.27711	18.80952	4.404762	28.92857	24.64286
2	8		0 g	757.375	228.125	15.25	51.75	11.2963	3.425926	25.92593	22.42718
2	48		579 g	516.7416	139.4382	8.202247	8.202247	11.40187	3.457944	14.76636	19.34579
2	48		0 g	630.8642	207.284	15.06173	9.012346	11.84466	3.592233	20.09709	20.09709
2	2		579 c	1408.9	416.1	32.9	292	76.7	15.8	97.3	105.9
2	2		0 c	1292.1	343.1	24.3	219	73	12.2	113.2	109.5
2	8		579 c	1262.9	343.1	20.7	219	69.4	12.2	105.9	105.9
2	8		0 c	1314	365	24.3	243.3	71.8	12.2	109.5	109.5
2	48		579 c	1481.9	438	36.5	413.7	101	12.2	146	133.8
2	48		0 c	1460	459.9	32.9	316.3	101	12.2	142.4	142.4

Trt= treatment; spp= species; g= grass (*D. abyssinica*); c=cotton

Appendix 6: List of publications

1) R M Wilkins and R. Kabanyoro (1997). Weed control with herbicides and hand-hoe weedingin cotton in Uganda. *Proceedings of an International Brighton Crop Protection Conference-Weeds,* pp659-660.

2) R Kabanyoro and R M Wilkins (1999). Integration of reduced dose rates of fluazifop-butyl or sethoxydim with hand-hoe weeding for the control of *D. abyssinica* and other weed species. *Proceedings of an International Brighton Conference-Weeds*, pp577-578.